LONG-TERM IMPLICATIONS OF CHRONIC RADIATION EXPOSURE: A GENOMICS STUDY OF TWO CANIDS REVEALS EVOLUTIONARY AND ECOLOGICAL IMPACTS OF A NUCLEAR DISASTER

by

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(Under the Direction of Stacey L. Lance)

ABSTRACT

Environmental contamination is one of the leading ways humans have impacted ecosystems globally. Growing human populations and resulting increases in energy demands have increased the contamination footprint of industry. This increase in industrial activity and energy production has, at times, introduced high levels of radionuclides into the environment that impact both humans and wildlife. Population persistence in such habitats relies on individuals' ability to acclimate and populations to adapt, yet the health effects and mechanisms of adaptation to elevated ionizing radiation are not well described. In this dissertation I examine genomic, transcriptomic, and parasite infection patterns in two canids, gray wolves and raccoon dogs, from the Chernobyl Exclusion Zone (CEZ) and build a new model system for examining the effects of elevated levels of ionizing radiation. Canids are sentinel species, and ideal model organisms for extrapolating from individual effects to ecological effects, conservation implications, and human health effects. Here I describe population differentiation between wolves from the CEZ and those from northern Belarus. This differentiation, in addition to the negative impacts of elevated

radiation exposure, suggest radiation in the CEZ may act as a selective force driving population level differences. To explore individual level implications of exposure, I also describe gene and endogenous retroviral expression patterns which identify regulatory differences associated with immune responses, DNA repair, and cell homeostasis. These patterns are associated with CEZ residency as well as internal radiocesium activity rates. Specifically, multiple genes and genome regulatory patterns are associated with oncogenesis. Interestingly I identified cellular homeostasis and DNA damage genes as genomic regions and candidate genes under selection. Additionally, micro- and macroparasite infection rates within raccoon dogs and wolves from the CEZ help describe the complexity of ecological interactions within a highly contaminated environment. Parasite diversity and prevalence differed between host, parasites, and radiation exposure rates, with only herpes virus and canine parvovirus showing positive correlations with internal radiation activity. Collectively, this work highlights the importance of immune responses, viral infections, and oncogenesis in both detrimental responses as well as potentially stimulating mitigating responses to radiation exposure.

INDEX WORDS: regulatory divergence; signatures of selection; candidate genes; transcriptomics; environmental contaminants; canids; wolves; raccoon dogs; microparasite; macroparasite; endogenous retrovirus; genomic structure; SNP; Fst outliers; ionizing radiation; Chernobyl

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DEDICATION

I dedicate this dissertation to my dear family and friends who have encouraged me every day of this crazy journey. They continue to teach and inspire me to be curious about the world each and every day.

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CHAPTER 1

INTRODUCTION

We are currently in the proposed Anthropocene epoch (Subramanian, 2019), where human activity has become the dominating force shaping the structure and function of earth's ecosystems (Vitousek et al., 1997). Contamination from industrial operations is one of the leading manners which humans impact ecosystems and is of significant concern to human health (Berg et al., 2001; Prasad, Cole, & Hasse, 2004; Reilly, 2003; World Health Organization, 2006), especially in under-resourced communities where a disproportionate amount of pollution is concentrated (Brook, 1998; Bullard & Wright, 1993; Lewis, Hoover, & MacKenzie, 2017). With a perpetually growing human population, the demand for energy production will continue to impact landscapes. Searches for alternative energy solutions has led to nuclear power becoming a popular alternative energy solution with reactors being built worldwide (Gagarinskii et al., 2005; World Nuclear Association, 2019).

While the physical footprints of existing nuclear reactors are significant, the realized footprints are even larger given routine and unexpected releases of waste. Although there are strict protocols to protect human health and maximize environmental safety from radiation contamination associated with nuclear energy production, there is always a threat of nuclear disaster, as seen in Three Mile Island, Chernobyl and Fukushima. With many known benefits and ambiguous costs, nuclear energy remains controversial (Poumadère, Bertoldo, & Samadi, 2011; Shkvyria & Vishnevskiy, 2012). The ecological and human health impacts of disasters can

be devastating and costly, thus it is critical to more thoroughly understand the implications of radiation exposure on surrounding communities and environments.

Most of what we know about radiation exposure comes from acute exposure studies in human and lab models, however we know far less about the effects of chronic low dose exposure which is the most common form (Galván et al., 2014; Real et al., 2011). All organisms experience some degree of radiation exposure, either from natural or anthropogenic sources. Exposure from natural sources such as UV radiation, Uranium deposits, and radon releases occur regularly. However, the radiation exposure levels observed around nuclear test sites or nuclear reactors is at times magnitudes higher than any natural exposure occurring in the wild. Ionizing radiation from environmental sources has been shown to have significant effects at the individual (Ellegren et al., 1997; Møller, 2002; Morley, 2012) and community levels (Møller & Mousseau, 2013; Møller, Barnier, & Mousseau, 2012; Romanoskaya et al., 1998). Yet mechanisms driving ecological changes or adaptation to these environments remain largely unexplored.

Renewed interest in radioecological studies suggests that examining individual level dose responses in sentinel species, particularly while exploring ecologically relevant endpoints *in situ*, are needed to better understand the lasting implications of ionizing radiation exposure (Rhodes et al., 2020). To better understand the ecological and long-term health implications of radiation exposure we need a more appropriate model. Most of the work on chronic exposure has focused on birds (Einor et al., 2016; Møller & Mousseau, 2003; Ruiz-Rodriguez et al., 2017), small mammals (Jernfors et al., 2018; Kesäniemi et al., 2019; Matson et al., 2000), and invertebrates (Møller & Mousseau, 2018; Møller, 2002). Further, most previous studies have been challenged due to questions concerning methods, analyses, and estimation of exposure levels (Beresford & Copplestone, 2011; J. T. Smith, 2008). Studies often generalize radiation exposure to regional

environmental exposure estimates, despite the wide heterogeneous range of radiation described across the CEZ and individual dosimetry based studies are needed to describe true impacts of exposure (Smith J. T., 2008). Additionally, mammals are one of the most radiosensitive taxa (Whicker & Schultz, 1982), and long-lived mammals can bioaccumulate high internal levels of radioactivity (Møller & Mousseau, 2011) and potentially experience genotoxic impacts of exposure. There is a critical needed for longer-lived model species with robust genomic resources.

The physiological mechanisms underlying an individual's response to contaminant exposure are numerous and often result in subtle differences difficult to ascertain in wild free-living individuals. Gene expression pathways associated with contaminant exposure allow for fine-scale investigation of these mechanisms as well as allow for the exploration of inter-individual variation in response to contaminant exposure. Exposure to some contaminants, such as radionuclides, can result in genomic instability (Morgan, 2003) leading to increased mutation rates and detrimental health effects associated with altered genetic and epigenetic responses (Lourenço et al., 2013; Mavragani et al., 2017; Theodorakis et al., 2001). Not only can these effects be passed from cell to cell but also from parent to offspring, thereby resulting in numerous potential transgenerational effects (Ellegren et al., 1997; Pogribny et al., 2004; Tsyusko et al., 2007). With a better understanding of the genomic and regulatory patterns of individuals exposed to ionizing radiation, we can make sound predictions concerning the physiological impacts that can affect survival and reproduction.

In this dissertation I explore ecological and evolutionary impacts of long-term ionizing radiation exposure as a result of a nuclear disaster while using two canid species, gray wolf (*Canis lupus*) and raccoon dog (*Nyctereutes procyonoides*), as new model species.

Understanding the mechanisms underlying these impacts across a variety of species is critical in predicting and mitigating potentially devastating consequences of radiation accidents in the future.

Study system

The Chernobyl disaster occurred in 1986, when the nuclear power reactor released thousands of tons of radioactive material into the atmosphere and surrounding environment, devastating local villages and neighboring ecosystems. Radionuclides were deposited in a heterogeneous manner across the landscape due to wind and rain patterns (Smith & Beresford, 2005). The highest radiation concentrations fell in modern day Ukraine and Belarus, yet radioactive material fell across most of Europe and the Union of Soviet Socialist Republics. Following the disaster, a 4,762 km^2 exclusion zone, the Chernobyl Exclusion Zone, (CEZ) was established around the most highly contaminated region, and more than 200,000 people were evacuated from the most contaminated areas. This resulted in a region solely inhabited by wildlife, which experience highly increased levels of environmental ionizing radiation exposure to this day.

To date, most ecological work has been restricted to the Ukrainian side of the CEZ, leaving many ecological effects of the disaster unexplored across the larger and highly contaminated Belarussian region. This Belarussian portion of the CEZ is managed by the Polesye State Radiation Ecological Reserve (PSRER) and hosts wide spatial heterogeneity in radiation contamination distribution (soil contamination levels of 40 – >7000 kBq/m2). Further, the Ukrainian portion of the CEZ has maintained significant human impact through rebuilding of the sarcophagus and recent tourism operations, the PSRER has very minimal human impact. The combination of a wide contaminant gradient, lack of human activity, and diverse animal

communities provides an ideal habitat to investigate the long-term implications of living in a radioactively contaminated landscape.

To incorporate samples from a region with background levels of environmental radiation exposure levels, I collaborated with researchers and hunting organizations in the north of Belarus (BLR). This region of Belarus is sparsely inhabited by humans and consists of mixed hard wood forests intermixed with freshwater systems, similar to habitats experienced in the CEZ.

Chapter Summaries

In this dissertation, I used the CEZ as a model system to explore long-term evolutionary and ecological impacts of ionizing radiation contamination. In particular I examine genomic signatures of population differentiation, genomic and regulatory divergence, parasite prevalence, and endogenous retroviral activation with relation to radiation exposure in the CEZ.

Chapter 2

In chapter two, I examine different genomic attributes of gray wolf populations, including descriptions of population structure and gene regulatory networks in wolves exposed to a range of radiation in the CEZ. Here I examine RNA sequencing data of blood samples collected from wild free-ranging wolves from the CEZ and BLR. Importantly, I find strong indication of population differentiation between the CEZ and BLR. This is critical because it establishes that the wolves within the CEZ are likely a genetically distinct population that may have experienced selection over the past few decades. Additionally, to explore regulatory divergence in the CEZ, I analyze individual gene expression patterns, gene co-expression module architecture, and regulatory signatures of selection in modules and pathways altered by radiation exposure. Lastly, to examine possible physiological implications of chronic environmental radiation exposure, I

examined regulatory patterns across individual genes, gene modules, and pathways of interest associated internal radiocesium activity within the CEZ.

Chapter 3

In chapter three, I examine patterns of parasite prevalence and intensity in both gray wolves and raccoon dogs to assess potential health impacts associated with exposure to radiation. I employ multiple techniques including fecal floatation, seroprevalence, quantitative PCR, and herpesvirus transcription rates to identify and describe >30 parasites in the wolves and raccoon dogs from the CEZ and BLR. Here I describe parasite diversity and prevalence is generally not related to radiation exposure, however two viruses did show a significant correlation with internal radiocesium activity. These data help not only help describe the ecological differences between the radiation contaminated CEZ and the reference region of BLR, but also starts to explore long term health implications of environmental ionizing radiation exposure in these two canids from the CEZ.

Chapter 4

In chapter four, I examine endogenous retrovirus activation patterns in wolves experiencing a gradient of ionizing radiation contamination. I find that wolves from the CEZ have significantly more expressed endogenous retrovirus elements than those from BLR. Further, within the CEZ, expression is positively correlated with internal radiocesium activity. I also find evidence of interactions of endogenous retroviral activity and immune response and oncogenic genes, as well as immune function and cancer related pathways.

Chapter 5

In chapter five I examine genomic and regulatory patterns in raccoon dogs inhabiting the CEZ and BLR. Here I utilize RNA sequencing data to build a *de novo* transcriptome and

describe individual differential gene expression patterns associated with immune and cellular homeostasis. To describe potentially adaptive regulatory patterns in raccoon dogs from the CEZ I examine individual gene and gene co-expression module architecture and discuss potential ecological and natural history characteristics driving differences in regulatory patterns found in wolves (Chapter 2) and those described in raccoon dogs.

Chapter 6

In chapter six, I summarize my dissertation findings and go on to discuss directions for future research. I also reflect on how this work contributes to a general understanding of mechanisms driving ecological and evolutionary shifts observed in radiation contaminated landscapes.

REFERENCES

Beresford, N. A., & Copplestone, D. (2011). Effects of ionizing radiation on wildlife: what knowledge have we gained between the Chernobyl and Fukushima accidents? *Integrated Environmental Assessment and Management*, *7*(3), 371–373. https://doi.org/10.1002/ieam.238

Berg, M., Tran, H. C., Nguyen, T. C., Pham, H. V., Schertenleib, R., & Giger, W. (2001). Arsenic contamination of groundwater and drinking water in Vietnam: A human health threat. *Environmental Science and Technology*, *35*(13), 2621–2626. https://doi.org/10.1021/es010027y

Brook, D. (1998). Environmental Genocide: native Americans and Toxic Waste. *American Journal of Economics and Society*, *57*(1).

Bullard, R. D., & Wright, B. H. (1993). *Environm Ental Justice for All : Communi Ty*. *9*(5), 821–

841.

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

Ellegren, H., Lindgren, G., Primmer, C. R., & Møller, A. P. (1997). Fitness loss and germline mutations in barn swallows breeding in Chernobyl. *Nature*, *389*(6651), 593–596. https://doi.org/10.1038/39303

Gagarinskii, A. Y., Ignat'ev, V. V., Ponomarev-Stepnoi, N. N., Subbotin, S. A., & Tsibul'skii, V. F. (2005). Role of nuclear power in world energy production in the 21st century. *Atomnaya Energiya*, *99*(5), 323–336.

Galván, I., Bonisoli-Alquati, A., Jenkinson, S., Ghanem, G., Wakamatsu, K., Mousseau, T. A., & Møller, A. P. (2014). Chronic exposure to low-dose radiation at Chernobyl favours adaptation to oxidative stress in birds. *Functional Ecology*, *28*(6), 1387–1403. https://doi.org/10.1111/1365-2435.12283

Jernfors, T., Kesäniemi, J., Lavrinienko, A., Mappes, T., Milinevsky, G., Møller, A. P., … Watts, P. C. (2018). Transcriptional Upregulation of DNA Damage Response Genes in Bank Voles (Myodes glareolus) Inhabiting the Chernobyl Exclusion Zone. *Frontiers in Environmental Science*, *5*(January), 1–8. https://doi.org/10.3389/fenvs.2017.00095

Kesäniemi, J., Lavrinienko, A., Tukalenko, E., Mappes, T., Watts, P. C., & Jurvansuu, J. (2019). Infection load and prevalence of novel viruses identified from the bank vole do not associate with exposure to environmental radioactivity. *Viruses*, *12*(1). https://doi.org/10.3390/v12010044

Lewis, J., Hoover, J., & MacKenzie, D. (2017). Mining and Environmental Health Disparities in

Native American Communities. *Current Environmental Health Reports*, *4*(2), 130–141. https://doi.org/10.1007/s40572-017-0140-5

Lourenço, J., Pereira, R., Gonçalves, F., & Mendo, S. (2013). Metal bioaccumulation, genotoxicity and gene expression in the European wood mouse (Apodemus sylvaticus) inhabiting an abandoned uranium mining area. *Science of the Total Environment*, *443*, 673–680. https://doi.org/10.1016/j.scitotenv.2012.10.105

Matson, C. W., Rodgers, B. E., Chesser, R. K., & Baker, R. J. (2000). Genetic Diversity of Clethrionomys Glareolus Populations From Highly Contaminated Sites in the Chornobyl Region, Ukraine. *Environmental Toxicology and Chemistry*, *19*(8), 2130–2135. https://doi.org/10.1897/1551-5028(2000)019<2130:GDOCGP>2.3.CO;2

Mavragani, I. V., Nikitaki, Z., Souli, M. P., Aziz, A., Nowsheen, S., Aziz, K., … Georgakilas, A. G. (2017). Complex DNA damage: A route to radiation-induced genomic instability and carcinogenesis. *Cancers*, *9*(7), 1–22. https://doi.org/10.3390/cancers9070091

Møller, A. P., & Mousseau, T. A. (2013). Assessing effects of radiation on abundance of mammals and predator-prey interactions in Chernobyl using tracks in the snow. *Ecological Indicators*, *26*, 112–116. https://doi.org/10.1016/j.ecolind.2012.10.025

Møller, A. P., & Mousseau, T. A. (2003). Mutation and Sexual Selection: a Test Using Barn Swallows From Chernobyl. *Evolution*, *57*(9), 2139. https://doi.org/10.1554/03-051

Møller, A.P., & Mousseau, T. A. (2018). Reduced colonization by soil invertebrates to irradiated decomposing wood in Chernobyl. *Science of The Total Environment*, *645*, 773–779. https://doi.org/10.1016/j.scitotenv.2018.07.195

Møller, A. P. (2002). Developmental instability and sexual selection in stag beetles from Chernobyl and a control area. *Ethology*, *108*(3), 193–204. https://doi.org/10.1046/j.1439-

0310.2002.00758.x

Møller, A. P., Barnier, F., & Mousseau, T. A. (2012). Ecosystems effects 25 years after Chernobyl: pollinators, fruit set and recruitment. *Oecologia*, *170*(4), 1155–1165. https://doi.org/10.1007/s00442-012-2374-0

Møller, A. P., & Mousseau, T. A. (2011). Efficiency of bio-indicators for low-level radiation under field conditions. *Ecological Indicators*, *11*(2), 424–430. https://doi.org/10.1016/j.ecolind.2010.06.013

Morgan, W. F. (2003). Non-targeted and delayed effects of exposure to ionizing radiation: II. Radiation-induced genomic instability and bystander effects in vivo, clastogenic factors and transgenerational effects. *Radiation Research*, *159*(5), 581–596. https://doi.org/10.1667/RRAV19.1

Pogribny, I., Raiche, J., Slovack, M., & Kovalchuk, O. (2004). Dose-dependence, sex- and tissue-specificity, and persistence of radiation-induced genomic DNA methylation changes. *Biochemical and Biophysical Research Communications*, *320*(4), 1253–1261. https://doi.org/10.1016/j.bbrc.2004.06.081

Poumadère, M., Bertoldo, R., & Samadi, J. (2011). Public perceptions and governance of controversial technologies to tackle climate change: Nuclear power, carbon capture and storage, wind, and geoengineering. *Wiley Interdisciplinary Reviews: Climate Change*, *2*(5), 712–727. https://doi.org/10.1002/wcc.134

Prasad, K. N., Cole, W. C., & Hasse, G. M. (2004). Health Risks of Low Dose Ionizing. *Society for Experimental Biology and Medicine*, 378–382.

Real, A., Horemans, N., Newsome, L., Oudalove, A., Stark, K., Willrodt, C., … Hinton, T. G. (2011). FREDERICA effects database update within the EMRAS-II programme:

Contributing to evaluate the environmental impact of ionizing radiation. *Radioprotection*, *46*(6), S695–S697.

Reilly, C. (2003). Metal Contamination of Food: Its Significance for Food Quality and Human Health. In *Nutrition Bulletin* (Vol. 28). https://doi.org/10.1046/j.1467-3010.2003.00356.x

Rhodes, O. E., Bréchignac, F., Bradshaw, C., Hinton, T. G., Mothersill, C., Arnone, J. A., … Zimmerman, J. K. (2020). Integration of ecosystem science into radioecology : A consensus perspective. *Science of the Total Environment*, *740*, 140031. https://doi.org/10.1016/j.scitotenv.2020.140031

Romanoskaya, V. A., Sokolov, I.G., Rokitko, R.V., & Chernyay, N.A. (1998). Effect of radioactive contamination on soil bacteria in the 10-km zone around the Chernobyl nuclear power plant. *Microbiology*, *67*(2), 226–231.

Ruiz-Rodriguez, M., Møller, A. P., Mousseau, T. A., & Soler, J. J. (2017). Capacity of blood plasma is higher in birds breeding in radioactively contaminated areas. *PLoS ONE*, *12*(6), 1–12. https://doi.org/10.1371/journal.pone.0179209

Shkvyria, M., & Vishnevskiy, D. (2012). Large carnivores of the chernobyl nuclear power plant exclusion zone. *Vestnik Zoologii*, *46*(3), 21–28. https://doi.org/10.2478/v10058-012-0020-2

Smith, J., & Beresford, N. A. (2005). *Chernobyl Catastrophe and Consequences* (1st ed.). https://doi.org/10.1007/3-540-28079-0

Smith, J. T. (2008). Is Chernobyl radiation really causing negative individual and population-level effects on barn swallows? *Biology Letters*, *4*(1), 63–64; discussion 65-6. https://doi.org/10.1098/rsbl.2007.0430

Subramanian, M. (2019). Humans versus Earth: the quest to define the Anthropocene. *Nature*, *572*(7768), 168–170. https://doi.org/10.1038/d41586-019-02381-2

Theodorakis, C. W., Bickham, J. W., Lamb, T., Medica, P. a, & Lyne, T. B. (2001). Integration of genotoxicity and population genetic analyses in kangaroo rats (Dipodomys merriami) exposed to radionuclide contamination at the Nevada Test Site, USA. *Environmental Toxicology and Chemistry / SETAC*, *20*(2), 317–326. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11351431

Tsyusko, O., Yi, Y., Coughlin, D., Main, D., Podolsky, R., Hinton, T. G., & Glenn, T. C. (2007). Radiation-induced untargeted germline mutations in Japanese medaka. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, *145*(1), 103–110. https://doi.org/10.1016/j.cbpc.2006.08.010

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science*, *277*(5325), 494–499. https://doi.org/10.1126/science.277.5325.494

Whicker, F. W., & Schultz, V. (1982). Radioecology: Nuclear Energy and the Environment. *The Journal of Applied Ecology*, *21*(2), 733. https://doi.org/10.2307/2403460

World Health Organization. (2006). Health Effects of the Chernobyl Accident and Special Health Care Programmes. In *Report to the UN Chernobyl Forum Expert Group "Health."*

World Nuclear Association. (2019). World Energy Needs and Nuclear Power. Retrieved from https://www.world-nuclear.org/information-library/current-and-future-generation/world-energy-needs-and-nuclear-power.aspx#:~:text=In IEO-2016%2C nuclear power,2.3 PWh to 4.5 PWh.

CHAPTER 2

A NUCLEAR DISASTER'S LEGACY: MULTIGENERATIONAL EXPOSURE TO RADIATION IN THE CHERNOBYL EXCLUSION ZONE IS LINKED TO REGULATORY DIVERGENCE AND SELECTION IN GRAY WOLVES[1]

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ABSTRACT

We continue to discover the impacts human activity has on ecological and evolutionary processes. In particular, energy production has led to global contamination of landscapes, yet we still lack understanding of the mechanisms driving ecological and evolutionary shifts as a result of this contamination. Here we examine genomic and regulatory implications of a nuclear disaster on a wild free-ranging canid species, the gray wolf (*Canis lupus*). We find wolves from the Chernobyl Exclusion Zone are genetically differentiated and possess four polymorphisms suggestive of genomic selection at genes associated with DNA and cell damage. Additionally, we find regulatory divergence between wolves with and without exposure to radiation, with evidence of adaptive regulatory evolution in gene co-expression modules associated with internal radiocesium activity. Further, immunological and oncogenic related pathways exhibit differential regulation in wolves from the Chernobyl Exclusion Zone. This study identifies genomic and regulatory mechanisms of local adaptation to ionizing radiation exposure as a result of a nuclear energy disaster.

INTRODUCTION

Humans have become such a dominating force in shaping the structure and function of Earth's ecosystems (Vitousek et al., 1997) that a new Anthropocene epoch has been proposed (Subramanian, 2019). Environmental contamination from industrial operations is one of the leading ways habitats are affected and is of concern to both human and ecosystem health (Berg et al., 2001; Henderson et al., 2012; Møller et al., 2012; Prasad, Cole, & Hasse, 2004; Reilly, 2003; World Health Organization, 2006). Growing human populations continue to increase demand for resources and energy production and propel the search for additional low-carbon energy sources.

Nuclear power offers a low emission option, but it remains controversial due to the potential for nuclear accidents, as seen in extreme at Three Mile Island, Chernobyl and Fukushima. Scientific and public controversy remains over the ecological implications of radiation contaminated landscapes (Beresford & Copplestone, 2011; Smith, 2008). For wildlife populations, these landscapes can be attractive refugia that appear as suitable habitat, but in which survival, reproduction, and/or development are negatively affected (Møller et al., 2005; Willson & Hopkins, 2013).

The Chernobyl disaster was one of the largest releases of radioactive material into the environment and is arguably the most well studied nuclear accident. Some research suggests the Chernobyl Exclusion Zone (CEZ) may act as one of the largest ecological sinks ever observed (Møller et al., 2012), and that there is reduced diversity, survival, and abundance of wildlife in the surrounding contaminated habitat (Møller & Mousseau, 2011). Yet, recent research has identified diverse and abundant mammal communities within the CEZ (Deryabina et al., 2015; Schlichting etal., 2019; Webster et al., 2016).

All organisms are exposed to sources of radiation from natural sources such as UV radiation, Uranium deposits, and radon releases. Thus, ionizing radiation is not a novel exposure and species are likely to possess mechanisms of coping with exposure. However, the radiation exposure levels observed around nuclear test sites or nuclear accidents is at times magnitudes higher than any natural exposure occurring in the wild. Ionizing radiation from natural sources or nuclear accidents can be genotoxic (Ellegren et al., 1997; Møller & Mousseau, 2015) and alter individual morphology (Møller, 2002), population density (Møller & Mousseau, 2013), and community composition (Møller et al., 2012; Romanoskaya et al.,1998). Consequently, in habitats such as the CEZ, the ecological impacts likely influence individual variation in survival

and reproductive success and may have led to selection at adaptive loci, and/or changes in genome wide genetic diversity (Bickham, 2011). Thus, populations inhabiting the CEZ provide a unique model for examining adaptation to environmental contaminants.

Typically, the effects of contaminant exposure are based on population models constructed to infer how individual level effects translates to population level impacts (Bickham, 2011). Recent examinations suggest that current ecological risk assessment models are failing to protect biodiversity and that additional data are needed. In particular, for radiological exposure we need data that link causal mechanisms of radiation impacts at the individual level to quantitative metrics that reflect ecosystem functions sensitive to radiological contaminants (Rhodes et al., 2020). Single organism assessments are informative in this endeavor, particularly when focusing on sentinel species (Rhodes et al., 2020).

Here we investigate the mechanisms by which exposure to radiation can drive ecological and evolutionary change in a wild free-ranging sentinel species, the gray wolf (*Canis lupus*). Mammals are one of the most radiosensitive taxa (Whicker & Schultz, 1982), and long lived mammals can bioaccumulate high internal levels of radioactivity (Møller & Mousseau, 2011) while also potentially accumulating detrimental genotoxic impacts of exposure. The gray wolf is an ideal model for this system given its longer life span than species previously examined, high trophic level, and because its close relative, the domestic dog, is a focal species for understanding environmental influences on human health and oncogenesis. We utilize the robust genomic data from the domestic dog to explore signatures of selection to elevated ionizing radiation levels in gray wolves. We address genomic signatures of population structure and candidate genes under selection, while also examining individual and gene network expression

patterns to gain a better understanding of mechanisms and gene targets of radiation induced selection.

METHODS

Study site description and sample collection

In 1986, the Chernobyl nuclear reactor exploded, releasing over 45,300 kgs of ionizing radiation into the atmosphere. Much of the radiation settled over Eastern and Central Europe, with the highest concentrations falling in modern day Ukraine and southern Belarus (Wu et al., 2001). This large release of radioactive material was devastating to local villages and surrounding ecosystems. In an effort to mitigate the impacts of the irradiation, a 4,762 km^2 exclusion zone (CEZ) was established and more than 200,000 people were evacuated from the most highly contaminated regions. The Polesye State Radiation Ecological Reserve (PSRER) serves as the managing entity of the largest portion of the CEZ, found on the Belarus side, and hosts wide spatial heterogeneity in radiation contamination distribution (soil contamination levels of 40 – >7000 kilobecquerel (kBq)/m^2 Cs-137, Figure 1a). This contaminant gradient, a diverse mammal community (Deryabina, Kuchmel, Hinton, et al., 2015; Schlichting et al., 2019; S.C. Webster et al., 2016), and lack of human activity provide an ideal habitat to investigate the long-term implications of highly mobile species living in a radioactively contaminated landscape.

To incorporate reference samples from a region with background levels of radiation, we collaborated with hunting organizations in northern Belarus, ≤400 Km from the CEZ, to serve as a reference population (BLR) (Figure 1b). These samples were collected from sites characterized

by limited human activity, mosaics of mixed hardwood and coniferous forests, with freshwater systems dispersed throughout, and exhibiting habitat similar to what is found in the CEZ.

Internal radiocesium activity quantification

Lifetime radiocesium exposure was calculated differently depending on whether the sample was collected from live-trapped or hunted wolves. For wolves live-trapped within the CEZ, we conducted Cs-137 body burden counts using a portable Cd-Zn-Te spectroscopy system placed directly under the rear flank muscle as described in Hinton et al.(2019). For samples collected by hunting organizations, we quantified Cs-137 in lyophilized, homogenized muscle tissues using a Packard Cobra II auto-gamma counter (Model Cobra II 5003; Packard Instruments Co., Meriden, CT, USA) in a single 3-inch through-hole NaI detector. We used a ROI of 580e754 kiloelectron-Volts (keV) centered around 662 keV and conducted auto-calibration daily during the sample analysis using a traceable Cs-137 source (SREL-0113). We derived counter yield from matrix-specific standards quantities as described in Kennamer et al., (2017). We also conducted background corrected Cs-137 counts on each sample with 60 minutes counts. To assess minimum detectable concentrations (MDCs, Bq/g, dry mass) for each sample we followed methods described by Currie (1968). Lastly, we converted dry activity concentrations (Bq/g, dry weight) to wet activity concentrations (Bq/g, wet mass) using wet:dry tissue mass ratios.

Sample collection and preservation

We collected blood samples from the CEZ wolves (N = 9) during trapping events for a simultaneous study in the fall of 2014 (Hinton et al., 2019) and immediately transferred samples to RNAlater for stabilization of cellular RNA. Blood samples from BLR wolves (N = 9) were collected and preserved in RNALater immediately after wolves were hunted. After collection

and preservation, all samples were stored at -20°C until transfer to the University of Georgia Savannah River Ecology Lab where samples were transferred to -80°C until RNA isolation occurred.

RNA- sequencing & transcriptome filtering

For total RNA purification from blood samples we used the RiboPure Blood Kit (Life Technologies, 2011). We sequenced the full blood transcriptomes from 18 wolves with varying internal radiocesium activity (IRA) rates. Transcriptome library preparation and 150 bp paired-end sequencing were performed at the Georgia Genomics and Bioinformatics Core using Illumina NextSeq PE75 with all samples run across four lanes. We screened all reads for quality control using FastQC and filtered reads using Trimmomatic (Bolger, Lohse, & Usadel, 2014) to remove low quality reads and adapters. We then mapped quality controlled reads to the CanFam3.1 genome using a two-pass approach in STAR v.2.7.1a (Dobin et al., 2013) and quantified read counts with htseq-counts (Anders, Pyl, & Huber, 2015). Genes were considered expressed if they had ≥ 20 average counts. To identify differentially expressed genes we utilized edgeR (Robinson, McCarthy, & Smyth, 2009) while normalizing read counts by library size using the *cpm* function.

Identification of expression modules associated with internal radiation activity

We performed weighted gene correlation network analysis (WGCNA) using the WGCNA package in R (Langfelder & Horvath, 2008). To identify outlier samples, we compared mean pairwise correlations between samples. We then used a soft thresholding approach to approximate a scale free topological network to compare an adjacency matrix. Given our sample sizes and the low thresholding observed in our samples, we used the recommended power of 18 (Langfelder & Horvath, 2008). We then used topological overlap to create a cluster

dendrogram with signed correlations while implementing a minimum cluster size of 50 genes and merging closely correlated modules ($R^2 = 0.75$).

To confirm candidate modules associated with the CEZ (FDR < 0.05), we applied linear mixed effect models using module eigengenes and collection location and IRA. Given the potential for IRA to be confounded by sample location we also examined module associations specifically with IRA using linear models and only samples from within the CEZ. Once candidate modules were identified, we examined gene enrichment in modules of interest using gProfileR (Reimand et al., 2016) in R to identify gene ontology categories.

Identification of candidate genes under selection

To explore genomic regions exhibiting significant differentiation across the CEZ barrier, we utilized the quality controlled CanFam3.1 genome aligned transcriptome reads described above. SNPs were called from alignments with the GATK HaplotypeCaller and GenotypeGVCF (McKenna et al., 2010) using the recommended RNAseq parameters and with PCR duplicates removed with the MarkDuplicates function. We then filtered SNPs with VCFtools for a depth ≥ 10, quality ≥ 20, and reads with either < 20% missing data or no missing data. To perform population differentiation analysis we used the Bayesian clustering approach to perform assignment tests in FastSTRUCTURE 2.1 (Raj, Stephens, & Pritchard, 2014). We then calculated individual SNP statistics using the Populations program from Stacks v.2.52 (Catchen et al., 2013) and calculated SNP F_{st} values using the AMOVA method (Weir, 1996). To identify putative targets of selection, we examined the blood transcriptome for loci exhibiting significant differentiation (empirical $p < 0.001$) and differential gene expression in individuals from the CEZ.

Correlating internal radiocesium activity and transcription patterns

To explore patterns of expression within pathways associated with mitigating genotoxic and cellular damage as a result of radiation, we assessed transcription patterns within the CEZ wolves. We performed false discovery rate corrected spearman correlations between gene transcripts from each pathway and individual wolf IRA using *psych* package in R (Revelle, 2019).

RESULTS AND DISCUSSION

Population Structure

To explore genomic patterns of population differentiation in gray wolves from the CEZ, we examined structure between wolves from the CEZ and BLR, a reference population in northern Belarus ~400 Km from the CEZ (Figure 1a). We used RNA-sequencing data from 18 wolves, nine from each sampling location, to initially determine if these sites represented distinct genetic populations. Our Bayesian population structure analyses (Raj et al., 2014) clearly demonstrate CEZ and BLR to be separate populations with most wolves showing low signs of admixture and only one wolf from the CEZ exhibiting equal genomic ancestry from both populations, with 50% assigned to BLR and CEZ (Figure 1b). In addition, wolves from the CEZ had significantly higher IRA compared to those from BLR (Figure 1d. t-test: $t = 4.6472$, p-value = 0.00165), with some CEZ individuals exhibiting > 90 times the IRA as reference individuals. All of the wolves in this study were sampled within a geographic range of < 450 Km^2, much smaller than the 850 Km^2 sampling range that results in spatial autocorrelation for other European wolf populations (Hindrikson et al., 2017). Taken together, our results show elevated levels of radiation exposure experienced within the CEZ and clear population differentiation

between the two locations. This may suggest that radiation within the CEZ acts is acting as a selective pressure on CEZ populations, and could be indicative of local adaptation potentially resulting in a barrier to gene flow.

Regulatory Divergence Associated with the CEZ and IRA

To explore divergent regulatory patterns potentially contributing to adaptive responses to radiation, we next explored transcriptome wide patterns of gene expression divergence between CEZ and BLR wolves. Blood samples were collected from individuals during non-lethal trapping events for a separate study (Hinton et al., 2019). We used blood as the focal tissue both due to non-lethal sampling techniques and its key role in immune system responses that can be strongly affected by ionizing radiation exposure (Kusunoki & Hayashi, 2008; Manda et al., 2012) and impact individual health. In total, we identified 4,418 differentially expressed genes (Appendix material Figure 1a) and divergent global regulatory patterns (Appendix Figure 1b) between the CEZ and BLR wolves.

To assess additional gene expression patterns across the wolf blood transcriptomes, we used WGCNA (Langfelder & Horvath, 2008) to evaluate co-expression gene modules and identified eight co-expression modules within the blood transcriptome (identified as Modules 1–8, Figure 2). Each of these modules likely represents groups of genes which interact in the same functional/regulatory networks. Two of the identified modules, modules 1 and 6, were identified as significantly correlated with site of origin (CEZ vs BLR, Figure 2.2b; linear mixed effects model, $p < 0.025$), and module 3 was significantly correlated with IRA in wolves from the CEZ (Figure 2.2b and linear model, $p = 0.027$). Within these three modules of interest, we enriched 29 biological processes which associate with viral infections, cellular ion transport, immune system function, cellular apoptosis, DNA repair, and metabolic processes (Appendix Table 1).

Additionally, site of origin associated modules (modules 1 and 6) contained disproportionally more differentially expressed genes (Fisher's exact test: CEZ DE, p-value < 2.2e-16) as compared to the other modules. This suggests these modules may be involved in resistance to the negative impacts of radiation, potentially conferring mitigating effects of radiation exposure. Regulatory differences associated with collection location and IRA may be confounded by the strong relationship between IRA and location (Figure 1d). However, many of the biological processes highlighted in modules associated with collection location (modules 1 and 6) contain genes with dynamic or no dose response to IRA, with some genes increasing and maintaining expression at particular radiation exposure levels (Hu et al., 2005; Kusunoki & Hayashi, 2008), suggesting elevated IRAs may still drive divergent expression patterns within these modules.

Within module 3, the differentially expressed genes exhibited disproportionally higher module connectivity (t-test: t = 2.1559, df = 21.337, p = 0.04265) than the non-differentially expressed genes. More highly connected module genes are often transcriptional regulators such as transcription factors and thus may play an important role in gene regulation (Liu et al., 2019) and selection within regulatory networks. This connectivity bias and linear correlation with IRA implies possible regulatory adaptation within these networks.

Signatures of radiation induced regulatory divergence within adaptive pathways

After establishing differences in transcription patterns across a broad range of IRA, we focused on the CEZ wolves to assess fine scale pathway expression patterns correlation with IRA. We characterized select KEGG pathway expression by using the first principal components (PC1) of regulatory variation within each pathway and then tested for correlations between expression and IRA. To perform these analyses we selected six pathways based on their role in

maintaining genome integrity and mitigating cellular responses to ionizing radiation (Fritzell et al., 1997; Kriegs et al., 2010).

To assess potential signals of detrimental genotoxicity induced DNA damage repair and immune responses we examined base pair mismatch repair (KEGG: map03430) and cytokine-cytokine receptor interaction (KEGG: map04060) pathways. Additionally, genotoxicity and cellular damage stemming from ionizing radiation exposure can result in carcinoma development. So, to explore signals of oncogenesis in wolves from the CEZ, we examined pathway regulation associated with oncogenesis in four pathways (transcriptional misregulation in cancer (KEGG: map05202), chemical carcinogenesis (KEGG: map05204), chronic myeloid leukemia (KEGG: map05220), acute myeloid leukemia (KEGG: map05221). Each of these six pathways showed significant positive correlation with IRA (Appendix table 2, $p < 0.0002$), implying that individuals with higher IRAs also exhibit increased DNA damage and higher rates of oncogenesis.

Genomic signatures of selection

To assess candidate genes under selection, we conducted Analysis of Molecular Variance to calculate F_{ST} for each variable site (N = 7,989) between the CEZ and BLR wolf populations. We identified four genes located in the trailing edge of the distribution of these F_{ST} values (empirical $p < 0.001$, Figure 3a,b) that were significantly upregulated in CEZ wolves (ENSCAFG00000002154 "TLN1", ENSCAFG00000049795 "H2BC21", ENSCAFG00000017943 "ANXA6", ENSCAFG00000032529 "COTL1"). These genes are associated with platelet activation, Human T-cell leukemia virus 1 infection, Rap1 signaling pathway, and focal adhesion, which participate in cytoskeleton organization and cellular and DNA damage responses, well-recognized effects of radiation exposure (Kanehisa, 2019). Two of

these genes ("TLN1" and "COTL1") are actin assembly and binding genes that play an important role in cell migration and tumor growth (Godoy et al., 2013; Singel et al., 2013; Xia et al., 2018). Specifically, the Cotl1 protein is said to be a strong cancer-associated antigen and overexpression is shown to upregulate tumor-suppressor genes as well as inhibit the growth-promoting TGFβ pathway in humans (Xia et al., 2018). The TLN1 gene facilitates integrin interactions with extracellular matrixes and is associated with the release of acute myeloid leukemia cells from the bone marrow into circulation (Badie et al., 2016) and metastasis in cancers (Sakamoto et al., 2010). Annexin A6 (Anxa6) up regulation leads to activation of extracellular signal-regulated kinase and has been reported to be a PU.1 target in leukemic cells (Badie et al., 2016; Iseki et al., 2009). Lastly, the H2B clustered histone 21 (H2BC21) gene can be activated with herpes virus infection and is associated with viral carcinogenesis (Kanehisa, 2019). Interestingly, as a separate part of our study (Chapter 4) we find increasing herpesvirus transcription with increasing IRA in these wolves. Collectively, differential expression of these genes may reflect functional differences in immune and cellular maintenance systems.

CONCLUSION

This study presents evidence that contamination from the Chernobyl nuclear disaster may have led to regulatory divergence and adaptation within gene networks associated with internal radiocesium activity and identifies candidate genomic polymorphisms under selection in the long- lived gray wolf. The observed evidence of selection at genes associated with actin structure, cell migration, and histone regulation, suggest fundamental cell and genome structural apparatuses are at the center of mitigating radiocesium induced cell deformities. Additionally, gene module networks suggest virus and radiocesium interactions may be simultaneously driving

divergent patterns in immune function with increased radiocesium exposure, potentially with additive cellular apoptotic effects. Further study is needed to understand how hosts, viruses, and ionizing radiation interreact with host immune systems. As the blood tissue examined here is characterized by numerous cell types, particularly many facilitating immune function, these data represent a cumulative examination of expression across potentially variable cell compositions. Additional study is not only needed to assess differences in blood cell type with radiation exposure, but also to understand how divergent regulatory networks identified here influence individual variation in raidiocesium sensitivity. In conclusion, our findings suggest that environments affected by nuclear accidents can drive population structure and evolutionary responses at the genetic and regulatory level.

REFERENCES

Agate, L., Mariotti, S., Elisei, R., Mossa, P., Pacini, F., Molinaro, E., … Pinchera, A. (2008). Thyroid autoantibodies and thyroid function in subjects exposed to chernobyl fallout during childhood: Evidence for a transient radiation-induced elevation of serum thyroid antibodies without an increase in thyroid autoimmune disease. *Journal of Clinical Endocrinology and Metabolism*, *93*(7), 2729–2736. https://doi.org/10.1210/jc.2008-0060

Anders, S., Pyl, P. T., & Huber, W. (2015). HTSeq-A Python framework to work with high-throughput sequencing data. *Bioinformatics*, *31*(2), 166–169. https://doi.org/10.1093/bioinformatics/btu638

Badie, C., Blachowicz, A., Barjaktarovic, Z., Finnon, R., Michaux, A., Sarioglu, H., … Bouffler, S. D. (2016). Transcriptomic and proteomic analysis of mouse radiation-induced acute myeloid leukaemia (AML). *Oncotarget*, *7*(26), 40461–40480.

https://doi.org/10.18632/oncotarget.9626

Beresford, N. a, & Copplestone, D. (2011). Effects of ionizing radiation on wildlife: what knowledge have we gained between the Chernobyl and Fukushima accidents? *Integrated Environmental Assessment and Management*, *7*(3), 371–373. https://doi.org/10.1002/ieam.238

Berg, M., Tran, H. C., Nguyen, T. C., Pham, H. V., Schertenleib, R., & Giger, W. (2001). Arsenic contamination of groundwater and drinking water in Vietnam: A human health threat. *Environmental Science and Technology*, *35*(13), 2621–2626. https://doi.org/10.1021/es010027y

Bickham, J. W. (2011). The four cornerstones of evolutionary toxicology. *Ecotoxicology*, *20*(March), 497–502. https://doi.org/10.1007/s10646-011-0636-y

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Catchen, J. M., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. (2013). Stacks: an analysis tool set for population genomics. *Molecular Ecology*, *22*(11), 3124–3140. https://doi.org/10.1111/mec.12354.Stacks

Currie, L. A. (1968). Limits for Qualitative Detection and Quantitative Determination: Application to Radiochemistry. *Analytical Chemistry*, *40*(3), 586–593. https://doi.org/10.1021/ac60259a007

Deryabina, T. G., Kuchmel, S. V., Nagorskaya, L. L., Hinton, T. G., Beasley, J. C., Lerebours, A., & Smith, J. T. (2015). Long-term census data reveal abundant wildlife populations at Chernobyl. *Current Biology*, *25*(19), R824–R826. https://doi.org/10.1016/j.cub.2015.08.017

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., … Gingeras, T. R. (2013). STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics*, *29*(1), 15–21. https://doi.org/10.1093/bioinformatics/bts635

Ellegren, H., Lindgren, G., Primmer, C. R., & Møller, A. P. (1997). Fitness loss and germline mutations in barn swallows breeding in Chernobyl. *Nature*, *389*(6651), 593–596. https://doi.org/10.1038/39303

Fritzell, J. A., Narayanan, L., Baker, S. M., Bronner, C. E., Andrew, S. E., Prolla, T. A., … Glazer, P. M. (1997). Role of DNA mismatch repair in the cytotoxicity of ionizing radiation. *Cancer Research*, *57*(22), 5143–5147.

Godoy, P. R. D. V., Mello, S. S., Magalhães, D. A. R., Donaires, F. S., Nicolucci, P., Donadi, E. A., … Sakamoto-Hojo, E. T. (2013). Ionizing radiation-induced gene expression changes in TP53 proficient and deficient glioblastoma cell lines. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *756*(1–2), 46–55. https://doi.org/10.1016/j.mrgentox.2013.06.010

Henderson, B. L., Chumchal, M. M., Drenner, R. W., Deng, Y., Diaz, P., & Nowlin, W. H. (2012). Effects of fish on mercury contamination of macroinvertebrate communities of Grassland ponds. *Environmental Toxicology and Chemistry*, *31*(4), 870–876. https://doi.org/10.1002/etc.1760

Hindrikson, M., Remm, J., Pilot, M., Godinho, R., Vik, A., Baltr, L., … Ozolins, J. (2017). *Wolf population genetics in Europe : a systematic review , meta-analysis and suggestions for conservation and management*. *92*, 1601–1629. https://doi.org/10.1111/brv.12298

Hinton, T. G., Byrne, M. E., Webster, S. C., Love, C. N., Broggio, D., Trompier, F., … Beasley, J. C. (2019). GPS-coupled contaminant monitors on free-ranging Chernobyl wolves

challenge a fundamental assumption in exposure assessments. *Environment International*, *133*(July), 105152. https://doi.org/10.1016/j.envint.2019.105152

Hu, J., Kapoor, M., Zhang, W., Hamilton, S. R., & Coombes, K. R. (2005). Analysis of dose-response effects on gene expression data with comparison of two microarray platforms. *Bioinformatics*, *21*(17), 3524–3529. https://doi.org/10.1093/bioinformatics/bti592

Iseki, Y., Imoto, A., Okazaki, T., Harigae, H., & Takahashi, S. (2009). Identification of annexin 1 as a PU.1 target gene in leukemia cells. *Leukemia Research*, *33*(12), 1658–1663. https://doi.org/10.1016/j.leukres.2009.04.010

Kanehisa, M. (2019). Toward understanding the origin and evolution of cellular organisms. *Protein Science*, *28*(11), 1947–1951. https://doi.org/10.1002/pro.3715

Kennamer, R. A., Oldenkamp, R. E., Leaphart, J. C., King, J. D., Bryan, A. L., & Beasley, J. C. (2017). Radiocesium in migratory aquatic game birds using contaminated U.S. Department of Energy reactor-cooling reservoirs: A long-term perspective. *Journal of Environmental Radioactivity*, *171*, 189–199. https://doi.org/10.1016/j.jenvrad.2017.02.022

Kriegs, M., Kasten-Pisula, U., Rieckmann, T., Holst, K., Saker, J., Dahm-Daphi, J., & Dikomey, E. (2010). The epidermal growth factor receptor modulates DNA double-strand break repair by regulating non-homologous end-joining. *DNA Repair*, *9*(8), 889–897. https://doi.org/10.1016/j.dnarep.2010.05.005

Kusunoki, Y., & Hayashi, T. (2008). Long-lasting alterations of the immune system by ionizing radiation exposure: implications for disease development among atomic bomb survivors. *International Journal of Radiation Biology*, *84*(1), 1–14. https://doi.org/10.1080/09553000701616106

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network

analysis. *BMC Bioinformatics*, *9*. https://doi.org/10.1186/1471-2105-9-559

Life Technologies. (2011). RiboPure™-Blood Kit Protocol (PN 1928M Rev D). *Protocol*.

Liu, Y., Gu, H. Y., Zhu, J., Niu, Y. M., Zhang, C., & Guo, G. L. (2019). Identification of Hub Genes and Key Pathways Associated With Bipolar Disorder Based on Weighted Gene Co-expression Network Analysis. *Frontiers in Physiology*, *10*(August), 1–9. https://doi.org/10.3389/fphys.2019.01081

Manda, K., Glasow, A., Paape, D., & Hildebrandt, G. (2012). Effects of ionizing radiation on the immune system with special emphasis on the interaction of dendritic and T cells. *Frontiers in Oncology*, *2*(August), 1–9. https://doi.org/10.3389/fonc.2012.00102

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., … DePrist, M. A. (2010). The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, *20*(1297–1303), 1297–1303. https://doi.org/10.1101/gr.107524.110.20

Møller, A P, Bonisoli-Alquati, A., Rudolfsen, G., & Mousseau, T. A. (2012). Elevated mortality among birds in Chernobyl as judged from skewed age and sex ratios. *PLoS ONE*, *7*(4), 1–8. https://doi.org/10.1371/journal.pone.0035223

Møller, A. P, & Mousseau, T. A. (2011). Conservation consequences of Chernobyl and other nuclear accidents. *Biological Conservation*, *144*(12), 2787–2798. https://doi.org/10.1016/j.biocon.2011.08.009

Møller, A. P, Mousseau, T., Milinevsky, G., Peklo, A., Pysanets, E., & Szep, T. (2005). Condition, reproduction and survival of barn swallows from Chernobyl. *Journal of Animal Ecology*, *74*(6), 1102–1111. https://doi.org/10.1111/j.1365-2656.2005.01009.x

Møller, A. Pape. (2002). Developmental instability and sexual selection in stag beetles from

Chernobyl and a control area. *Ethology*, *108*(3), 193–204. https://doi.org/10.1046/j.1439-0310.2002.00758.x

Møller, A. P., Barnier, F., & Mousseau, T. A. (2012). Ecosystems effects 25 years after Chernobyl: pollinators, fruit set and recruitment. *Oecologia*, *170*(4), 1155–1165. https://doi.org/10.1007/s00442-012-2374-0

Møller, A. P., & Mousseau, T. A. (2011). Efficiency of bio-indicators for low-level radiation under field conditions. *Ecological Indicators*, *11*(2), 424–430. https://doi.org/10.1016/j.ecolind.2010.06.013

Møller, A. P., & Mousseau, T. A. (2013). Assessing effects of radiation on abundance of mammals and predator-prey interactions in Chernobyl using tracks in the snow. *Ecological Indicators*, *26*, 112–116. https://doi.org/10.1016/j.ecolind.2012.10.025

Møller, A. P., & Mousseau, T. A. (2015). Strong effects of ionizing radiation from Chernobyl on mutation rates. *Scientific Reports*, *35*(1), 1–10. https://doi.org/10.1038/srep08363

Prasad, K. N., Cole, W. C., & Hasse, G. M. (2004). Health Risks of Low Dose Ionizing. *Society for Experimental Biology and Medicine*, 378–382.

Raj, A., Stephens, M., & Pritchard, J. K. (2014). FastSTRUCTURE: Variational inference of population structure in large SNP data sets. *Genetics*, *197*(2), 573–589. https://doi.org/10.1534/genetics.114.164350

Reilly, C. (2003). Metal Contamination of Food: Its Significance for Food Quality and Human Health. In *Nutrition Bulletin* (Vol. 28). https://doi.org/10.1046/j.1467-3010.2003.00356.x

Reimand, J., Arak, T., Adler, P., Kolberg, L., Reisberg, S., Peterson, H., & Vilo, J. (2016). g:Profiler-a web server for functional interpretation of gene lists (2016 update). *Nucleic Acids Research*, *44*(W1), W83–W89. https://doi.org/10.1093/nar/gkw199

Revelle, W. (2019). *psych:Procedures for Psychological, Psychometric, and Personality Research* (pp. 0–386). pp. 0–386. Retrieved from https://cran.r-project.org/package=psych

Rhodes, O. E., Bréchignac, F., Bradshaw, C., Hinton, T. G., Mothersill, C., Arnone, J. A., … Zimmerman, J. K. (2020). Integration of ecosystem science into radioecology : A consensus perspective. *Science of the Total Environment*, *740*, 140031. https://doi.org/10.1016/j.scitotenv.2020.140031

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2009). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, *26*(1), 139–140. https://doi.org/10.1093/bioinformatics/btp616

Romanoskaya, V. A., Sokolov, I. G., Rokitko, R. V., & Chernyay, N. A. (1998). Effect of radioactive contamination on soil bacteria in the 10-km zone around the Chernobyl nuclear power plant. *Microbiology*, *67*(2), 226–231.

Sakamoto, S., McCann, R. O., Dhir, R., & Kyprianou, N. (2010). Talin1 promotes tumor invasion and metastasis via focal adhesion signaling and anoikis resistance. *Cancer Research*, *70*(5), 1885–1895. https://doi.org/10.1158/0008-5472.CAN-09-2833

Schlichting, P. E., Love, C. N., Webster, S. C., & Beasley, J. C. (2019). Efficiency and composition of vertebrate scavengers at the land-water interface in the Chernobyl Exclusion Zone. *Food Webs*, *18*. https://doi.org/10.1016/j.fooweb.2018.e00107

Singel, S. M., Cornelius, C., Batten, K., Fasciani, G., Wright, W. E., Lum, L., & Shay, J. W. (2013). A targeted RNAi screen of the breast cancer genome identifies KIF14 and TLN1 as genes that modulate docetaxel chemosensitivity in triple-negative breast cancer. *Clinical Cancer Research*, *19*(8), 2061–2070. https://doi.org/10.1158/1078-0432.CCR-13-0082

Smith, J. T. (2008). Is Chernobyl radiation really causing negative individual and population-

level effects on barn swallows? *Biology Letters*, *4*(1), 63–64; discussion 65-6. https://doi.org/10.1098/rsbl.2007.0430

Subramanian, M. (2019). Humans versus Earth: the quest to define the Anthropocene. *Nature*, *572*(7768), 168–170. https://doi.org/10.1038/d41586-019-02381-2

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science*, *277*(5325), 494–499. https://doi.org/10.1126/science.277.5325.494

Webster, S.C., Byrne, M. E., Lance, S. L., Love, C. N., Hinton, T. G., Shamovich, D., & Beasley, J. C. (2016). Where the wild things are: Influence of radiation on the distribution of four mammalian species within the Chernobyl Exclusion Zone. *Frontiers in Ecology and the Environment*, *14*(4). https://doi.org/10.1002/fee.1227

Webster, Sarah C, Byrne, M. E., Lance, S. L., Love, C. N., Hinton, W. G., Shamovich, D., & Beasley, J. C. (2016). Where the wild things are: influence of radiation on the distribution of four mammalian species within the Chernobyl Exclusion Zone. *Fronteirs in Ecology and the Environment*, *14*(4), 185–190.

Weir, B. (1996). *Genetic Data Analysis II: Methods for Discrete Population Genetic Data*. Sunderland, Mass, Sinauer Associates, 1996.

Whicker, F. W., & Schultz, V. (1982). Radioecology: Nuclear Energy and the Environment. *The Journal of Applied Ecology*, *21*(2), 733. https://doi.org/10.2307/2403460

Willson, J. D., & Hopkins, W. a. (2013). Evaluating the Effects of Anthropogenic Stressors on Source-Sink Dynamics in Pond-Breeding Amphibians. *Conservation Biology : The Journal of the Society for Conservation Biology*, *00*(0), 1–10. https://doi.org/10.1111/cobi.12044

World Health Organization. (2006). Health Effects of the Chernobyl Accident and Special

Health Care Programmes. In *Report to the UN Chernobyl Forum Expert Group "Health."*

Wu, L., Qin, Y. M., Huang, B., Zong, Z. M., Wei, X. Y., Chen, Q. R., & Zou, G. L. (2001). Environmental consequences of the Chernobyl accidenct and their remediation: twenty years of experience. *Wuhan University Journal of Natural Sciences*, *6*(4), 854–858.

Xia, L., Xiao, X., Liu, W. L., Song, Y., Liu, T. J. J., Li, Y. J., … Ben-David, Y. (2018). Coactosin-like protein CLP/Cotl1 suppresses breast cancer growth through activation of IL-24/PERP and inhibition of non-canonical TGFβ signaling. *Oncogene*, *37*(3), 323–331. https://doi.org/10.1038/onc.2017.342

FIGURES

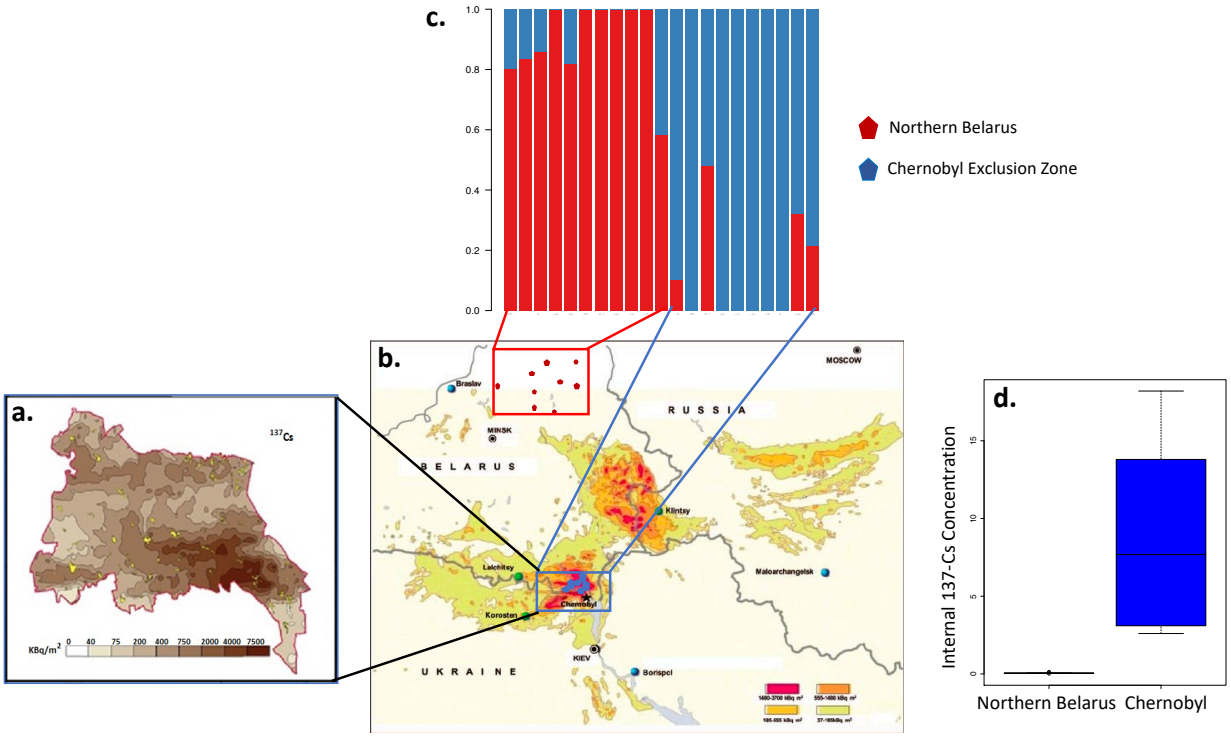

Figure 2.1: (a) PSRER within the Chernobyl Exclusion Zone and **(b)** the country of Belarus contain a gradient of ionizing radiation contamination. Wolves from northern Belarus (red points, N=9) and the Chernobyl Exclusion Zone (blue points, N = 9) represent **(c)** distinct genetic populations and those from the PSRER. **(d)** Wolves from the PSRER have significantly higher and more variable internal radiocesium (Cs-137) activity rates. (Maps adapted from Agate et al., 2008 and Hinton et al., 2019).

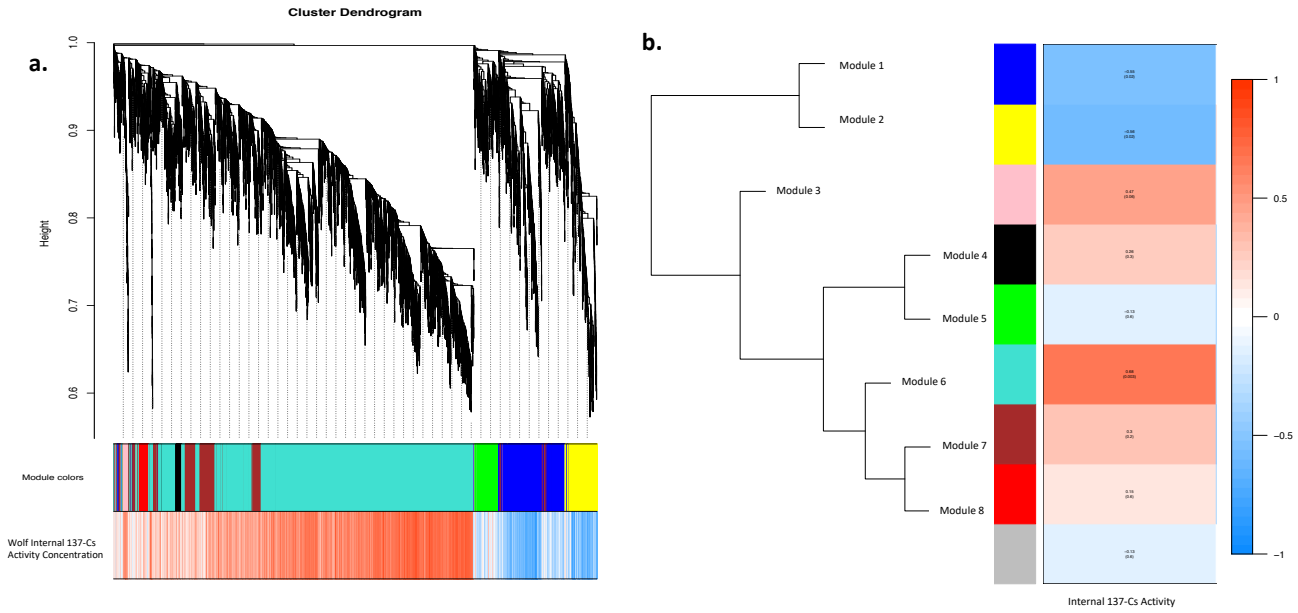

Figure 2.2: Regulatory modules associated with internal Cs-137 activity in wolves. (**a**) Gene expression correlation patterns within the gray wolf blood transcriptome depicted in a cluster dendrogram where each branch represents a single gene. Groups of highly correlated genes are then clustered and identified by Module Colors. Wolf internal Cs-137 Activity Concentration depicts correlation between each gene and individual variation in internal Cs-137 activity, red indicates a positive correlation and blue a negative correlation. (**b**) Hierarchical clustering dendrogram of each of the eight individual module eigengenes. The correlation of these eigengenes with internal radiocesium (Cs-137) activity rates is depicted in the heatmap, which is color-coded by correlation value and corresponds to the bar to the right of the graph. Each grid contains the eigengene correlation value with internal Cs-137 activity (top) and Pearson's R for significant correlations ($p < 0.05$, bottom).

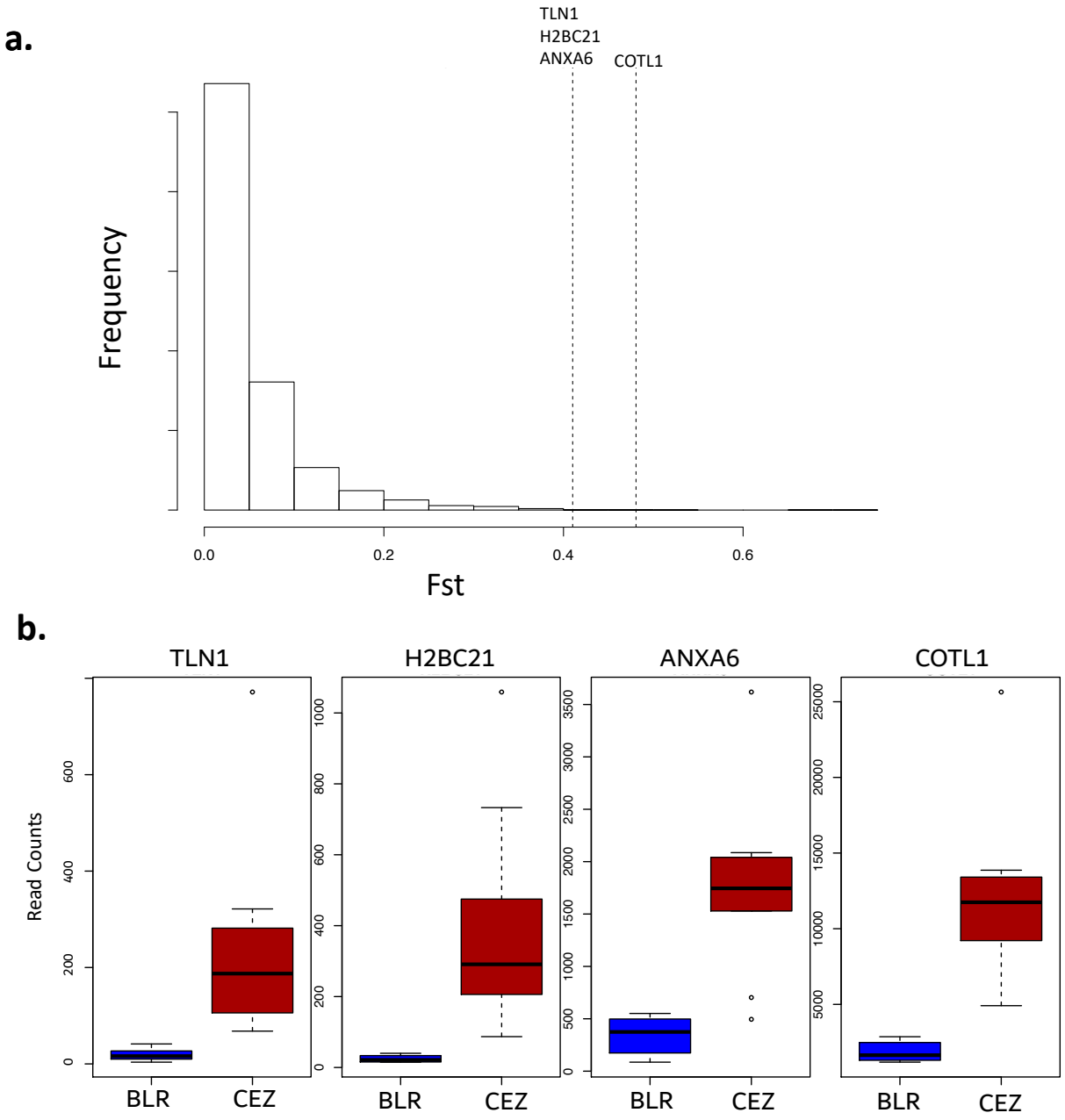

Figure 2.3: Genomic signals of candidate genes under selection (**a**) Assessment for genetic differentiation using an Fst outlier (<0.001) approach between wolves from the CEZ and BLR identified four candidate genes under selection. (**b**) Expression differences between CEZ and northern Belarus wolves at SNP Fst outliers in wolves from the Chernobyl Exclusion Zone.

CHAPTER 3

DIVERSITY AND PREVALENCE OF PARASITES IN TWO CANIDS, RACCOON DOGS AND GRAY WOLVES, FROM THE CHERNOBYL EXCLUSION ZONE[2]

[2] Love CN, SC Webster, D Shamovich, ME Byrne, PE Schlichting, JC Beasley, TG. Hinton, and SL Lance. To be submitted to *International Journal for Parasitology.*

ABSTRACT

Acute exposure to radiation is widely recognized to cause morbidity and mortality in wildlife as well as influence immune response and host-parasite interactions. However, robust information is lacking regarding the impacts of chronic radiation exposure on parasitism rates *in situ*. Radiation exposure can impact parasite prevalence and intensity in multiple ways, including altering host exposure to parasites and susceptibility, as well as parasite reproduction and infectivity. Here we examine parasite diversity and prevalence in gray wolves and raccoon dogs exposed to elevated levels of ionizing radiation in the Chernobyl Exclusion Zone. Carnivores are at higher risk for accumulation of contaminants due to their high trophic level and long life span, and thus provide good model species. We used fecal floatation to describe 11 gastrointestinal parasites and observed no difference in parasite diversity or prevalence between hosts or estimated radiation exposure. Serological testing showed evidence of *Borrelia burgdorfi, Dirofilaria immitis,* and canine parvovirus in wolves, with canine parvovirus significantly associated with internal radiocesium activity. We further examined 28 blood-borne parasite species using a qPCR technique in wolves or raccoon dogs and found no correlation of radiation exposure and parasite prevalence. Finally, we found clear correlations of Herpesviridae transcription and internal radiocesium activity. Collectively these data help describe the complexity of parasite infection interactions with elevated radiation exposure and these data inform future and ongoing examination of ecological impacts of chronic radiation exposure.

INTRODUCTION

Exposure to contamination can have numerous impacts on wildlife, including altering parasitism rates (Marcogliese et al., 2005; Marcogliese & Pietrock, 2011; Morley, Lewis, & Hoole, 2006). Most of this research has focused on contaminants associated with agricultural (Buck et al., 2015; King et al., 2010; Zuther et al., 1999) and industrial (Bichet et al., 2013; Love et al., 2016; Thielen et al., 2004) operations, but there has been a lack of work focusing on ionizing radiation, despite the ubiquitous nature of radiation exposure. While all wildlife experience radiation exposure naturally from UV radiation (Acevedo-Whitehouse & Duffus, 2009; Martin, 2001), some populations experience additional natural exposure from Uranium or radon deposits (Sazykina & Kryshev, 2006), and others are exposed to dramatically elevated levels from anthropogenic contamination (Lourenço, Mendo, & Pereira, 2016; Lourenço et al., 2013). Landscapes can be contaminated with ionizing radiation as a result of industrial practices, nuclear testing, or nuclear disasters (Eisenbud & Gesell, 1997). Exposure to radiation can cause sublethal effects on individual physiology (Bonisoli-Alquati et al., 2010; Møller et al., 2011) and result in altered life histories (Adamovich, 1998) and population densities (Møller & Mousseau, 2013). Concurrently, all organisms are subjected to infections from an array of parasites which can affect host ecology, immunology, and physiology and consequently may be a source of additional stress for organisms. Thus, exposure to radiation is likely to alter host-parasite interactions. In fact these host-parasite-environment interactions are increasingly recognized as regulating factors in how animals respond to contaminated environments (Morley, 2012). The interactive effects of elevated ionizing radiation exposure and parasitism rates are of increasing interest particularly as nuclear power is expanding worldwide .

One of the most likely ways exposure to ionizing radiation could impact rates of parasitism is through direct effects on host immune function and behavior. Animals living in radiation contaminated environments exhibit depressed immunity, possibly as a result of radiation-reduced immune function (Camplani, Saino, & Møller, 1999). Many immune response mechanisms can be affected by radionuclide exposure including the production of leukocytes (Gridley et al., 2001; Dainiak et al., 2003), antibodies (Agate et al., 2008), and cytokines (Schröder et al., 2018). In some cases the immunological effects of ionizing radiation are consistent across exposure levels, such as attrition of T lymphocyte function and other key players in cell-mediated adaptive immune responses (Manda, Glasow, Paape, & Hildebrandt, 2012). However, some inflammatory responses, such as macrophage activity, show stimulation at low exposures (<0.1 Gray (Gy)) of radiation and suppression at high exposures (> 2 Gy) (Liu, 2003). In the wild, birds in the Chernobyl Exclusion Zone have decreased immune function (Camplani et al., 1999), depressed leukocyte and immunoglobulin levels and smaller spleens (Camplani et al., 1999), and show ranges of specific antimicrobial activity of individual hosts and radiation levels (Ruiz-Rodriguez, Møller, Mousseau, & Soler, 2017).

Aside from impacting host immune function, there are many ways that ionizing radiation could affect parasite transmission rates. A change in the health of a host could alter feeding and movement patterns leading to changes in contact rates between hosts and pathogens. This may be caused by either increased or decreased contact between hosts and/or changes in the production and deposition of contaminated bodily fluids (i.e. mucus, feces, or vomit). For example, exposure to radiation can cause *Daphnia* species to alter feeding and shoaling behavior (Mothersill et al., 2006) and decreases activity in neonatal rats (Wallace, Daniels, & Altman, 1972). Additionally, dramatic ecological shifts have been observed in natural environments

following nuclear disasters. After the Chernobyl disaster, plant (Mousseau et al., 2013), invertebrate (Møller & Mousseau, 2009), and vertebrate (Møller & Mousseau, 2013) communities changed and decomposition rates were altered (Bonzom et al., 2016; Mousseau et al., 2014). These community level changes can affect resource availability across a landscape and drive changes in host movement patterns. This was seen in barn swallows from Chernobyl who dispersed further when foraging than those in reference regions (Møller et al., 2006). Altered resource availability and community structure can also affect indirect transmission via mechanical or biological vectors if host body condition and/or the presence and abundance of intermediate hosts is changed. All of these elements may impact host pathogen contact rates.

Radiation exposure may also influence host-parasite interactions through direct impacts on parasites. Helminths can bioaccumulate more radionuclides than their host species (Booth & Schulert, 1968; Nansen et al., 1976) and exhibit decreased survival and reproduction (Chai et al., 1995). In fact, irradiation experiments have shown decreased growth and host infection rates of *Angiostrongylus* larvae in humans (Ishii et al. 1986). Thus, in radiation contaminated regions helminth infection rates may be regulated by radionuclide accumulation in, and associated detrimental impacts on, the parasites. Altered virulence has also been observed with ionizing radiation exposure in protozoans. For example, *Trypanosoma gambiense* exhibits decreased growth and multiplication at low doses (12 kr, Halbertaedter 1938). *Trypanosoma cruzi* shows a similar initial reduction in growth, however resumes normal growth 120 hours post exposure. This resumption of growth is associated with a shift in protein isoform translation (Vieira et al., 2014), potentially suggesting rapid physiological adaptation to ionizing radiation exposure. This finding suggests that radiation may not only impact hosts' increased susceptibility and exposure

to pathogens, but also that hosts' increased parasite susceptibility may be negated by negative impacts of exposure on parasites.

There is renewed interest in the potential ecological impacts of ionizing radiation on the environment and a need to integrate ecological sciences with radioecology (Rhodes et al., 2020). The Chernobyl Exclusion Zone (CEZ) has served as an outstanding model for examining the effects of chronic radiation exposure on wildlife. However, there are limited field studies exploring parasite infection loads in regions with radiation contamination. There have been a few studies conducted in areas with naturally elevated background levels of radiation (Maslov 1967, 1972) or in areas surrounding Uranium mines (Waite et al 1988), but these areas have much lower levels of radiation than parts of landscapes contaminated with radiocesium due to nuclear reactor releases, such as the CEZ and Fukushima. Within the CEZ, Aguileta et al. (2016) found decreased prevalence of a plant fungal pathogen in the more highly contaminated regions, yet they did not observe differences in viability or fertility of the pathogen. In the only CEZ study of parasites in a mammalian host, Kesäniemi et al. (2019) found no evidence of altered viral prevalence, load, or virulence in voles as a result of environmental contamination level.

Given the detrimental impacts of radiation exposure described here on intermediate hosts, we might expect lower indirectly transmitted parasite infections in high radiation regions. Additionally, interactions of increased susceptibility and negative impacts of radiation observed in some gastrointestinal parasites (Chai et al.,1995) may suggest that we would not expect large differences in gastrointestinal parasite infection patterns. But, to better understand the potential impact of radiation exposure on rates of parasitism, we need data from wild hosts with individual level radiation accumulation exposure measurements. Previous field studies generalize radiation exposure to group or population exposure estimates, observing infection differences between

groups or populations, while not taking into account more precise individual level exposure estimates which may help describe individual exposure and endogenous factors influencing parasite infections. The aim of this study was to characterize a suite of macro- and micro-parasites with varying modes of transmission among two canids, the gray wolf (*Canis lupus)* and the raccoon dog (*Nyctereutes procyonoides)* to better understand infection patterns and potential health impacts to mammals inhabiting the CEZ.

METHODS

Site Description

The Chernobyl disaster occurred in 1986 devastating local human and wildlife communities and the surrounding ecosystems. The Chernobyl reactor explosion released over >45,000 kgs of radioactive material, much of which settled over a large portion of Europe and the Union of Soviet Socialist Republics, with the highest concentrations falling in what is now northern Ukraine and southern Belarus. In an effort to mitigate further damage from the disaster, a 4,762 km^2 exclusion zone (CEZ) was established around the reactor and the most highly contaminated regions, displacing more than 200,000 people. The area in Belarus is now managed by the Polesye State Radiation Ecological Reserve (PSRER) and is solely inhabited by wildlife and visiting PSRER employees who rotate their time on and off the CEZ. The soils within the PSRER have wide spatial heterogeneity in radiation contamination (soil contamination levels of 40 – >7000 kBq/m^2 Cesium-137 (Cs-137)). This contaminant gradient, a diverse mammal community (Dharmarajan et al., 2009; Schlichting et al., 2019; Webster et al., 2016), and lack of human activity in the zone provide an ideal habitat to investigate the long-term implications of highly mobile species living in a radioactively contaminated landscape.

We also included samples from a region with background levels of radiation. To do so, we collaborated with hunt clubs in a reference region in northern Belarus. The samples from this location were collected from sites characterized by habitats with similar forests and freshwater systems as what is found in the CEZ.

Sample Collection

We live trapped wolves and raccoon dogs using foothold traps across the contamination gradient found in the PSRER. Animal capture and handling was carried out in accordance with University of Georgia Animal Care and Use protocol A2015 05–004-Y2-A1. Ages were estimated upon trapping using tooth ware and wolf age estimates ranged from 1.5 - 9 years old. Raccoon dog estimated ages ranged from 1 – 5 years old. Wolf and raccoon dog samples were collected from both males and females. While processing trapped individuals, we collected blood samples, preserved them in RNAlater (Thermo Fisher Scientific) or RNAprotect (Qiagen), and stored samples at -20°C until transferred to the University of Georgia's Savannah River Ecology Lab, USA where they were stored at -80°C until processing. To obtain blood samples from hosts living in habitats with background radiation levels, we collaborated with hunting organizations in northern Belarus to collect fresh samples. These blood samples were collected and stored in the same manner as stated above. Muscle and liver tissue samples were also collected from these individuals and stored at -20°C.

For gastrointestinal parasite analysis, we collected fecal samples from wolves and raccoon dogs along road transects. Before initiating scat surveys, we cleared all scat samples from targeted roadways, and then performed surveys every five days for a total of six collection days (Gese, 1998). Only samples estimated to have been deposited within 24 hours of collection

were used for parasite quantification. Samples were stored at -20°C until parasite analyses were performed.

Radiocesium Quantification

We calculated host Cs-137 exposure differently depending on whether the sample was scat, blood from trapped wolves, or blood from hunted wolves. To estimate individual host radiation exposure for each scat sample, we calculated area-weighted means of soil Cs-137 contamination from a 1000m radius around each fecal collection location. UTM coordinate identified collection locations were referenced against geo-referenced CEZ Cs-137 soil contamination maps (Izrael and Bogdevich 2009; Webster et al. 2016). Geo-referenced Cs-137 soil contamination maps are described by eight radiation ranges, from the lowest radiation levels (40-75KBq/m2) to the highest radiation levels (≥7500 KBq/M2). We designed our transects to occur within regions with lower and higher levels of contamination and after collection we estimated the exposure rates from our scat samples. Based on these analyses we end up with two regions that we call the "low" (40-600Kbq/m2) and "high" (≥ 2,500 KBq/m2). radiation regions.

Samples tested for microparasite quantification were collected from both trapped and hunted wolves and raccoon dogs. For wolves and raccoon dogs in the CEZ we estimated calculated area-weighted means as described for the fecal samples above. These estimates were calculated from 50m areas around the trap location of each individual. For a subset of trapped individuals we directly estimated Cs-137 body burdens using a field Cd-Zn-Te spectroscopy system to calculate internal radiocesium activity for each individual as detailed in Hinton et al. (2019). We prepared muscle and liver samples for direct Cs-137 quantification from all hunted individuals. We lyophilized, homogenized, and packed each sample into a scintillation tube and recorded wet and dry mass values prior to and post sample preparation. Dry-tissue radiocesium

counting was performed on a Packard Cobra II auto-gamma counter (Model Cobra II 5003; Packard Instruments Co., Meriden, CT, USA) as described in Chapter 2 with a NaI detector and using a ROI of 580e754 kiloelectron-Volts (keV) centered approximately on 662 keV, with daily auto-calibration during analysis using a traceable Cs-137 source (SREL-0113). On each sample we performed 60 minute counts and 60 second background counts on blank scintillation tubes to conduct background corrected Cs-137 counts for each sample. Counter yield was derived from matrix-specific standards with known Cs-137 quantities (Kennamer et al., 2017). Minimum detectable concentrations (MDCs, Bq/g, dry mass) for each sample was determined according to methods described by Currie (1968). We then converted to wet activity (Bq/g, wet mass) from dry activity concentrations (Bq/g, dry weight) using wet:dry tissue mass ratios.

Gastrointestinal Parasite Identification and Quantification

We examined fecal samples microscopically for the presence of gastrointestinal parasite eggs, oocysts, and larvae by a standard flotation technique using Sheather's sugar solution (specific gravity 1.27). Briefly, we took 3 g thawed subsamples of each scat and rinsed them with distilled water before filtering samples using cheese cloth. We then mixed samples with 10 mL Sheather's sugar solution and centrifuged the samples in 15 mL conical tubes for 5 minutes at 1500 rpm. To collect parasite eggs and oocysts we placed a coverslip on the top of each tube during centrifugation. We then transferred the coverslip to a microscope slide for identification and counting of parasites. For each sample we quantified total egg or oocyst numbers and eggs/oocysts per gram feces.

To compare prevalence and co-infection rates of gastrointestinal parasites between wolves and raccoon dogs, we performed a Fisher's chi-square test. Parasite intensity (eggs/g) between individuals from areas with low and high levels of radiation was assessed with Mann-

Whitney *U*-test. We used general linear models to examine relationships between parasite intensity and radiation exposure.

Serological Testing

To identify antibodies to common canid infections we performed serological tests on the subset of wolves and raccoon dogs from the CEZ for which we had quantified Cs-137 body burdens (sample numbers Table 3.1). We screened for canine parvovirus and distemper using TiterCHEK CDV, CPV (Zoetis DIAGNOSTICS), and 6 vector borne pathogens (*Ehrlichia canis* and/or *E. ewingii*, *Borrelia burgdorferi*, *Anaplasmosis phagocytophilum* and/or *A. platys*, and *Dirofilaria immitis*) using an ELISA based test kit (IDXX 4Dx SNAP kit, IDEXX Laboratories, Inc.). All analyses were performed according to manufacturer's protocols and positives were identified according to the manufacturers standards. When more than one individual tested positive we conducted a Welch's t-test to test for significant differences in Cs-137 body burden between positive and negative individuals.

Blood-borne Parasite Quantification via qPCR

For whole blood stored in RNAlater we extracted RNA using the RiboPure Blood Kit (Thermo Fisher Scientific Inc.) and DNA using a modified Acid-Phenol technique. For whole blood stored in RNA Protect we extracted RNA and DNA using the RNeasy Protect Animal Blood Kit (QIAGEN Group) and DNeasy Blood and Tissue Kit (QIAGEN Group) respectively. We synthesized total cDNA from blood samples using 255 ng total RNA and the iScript cDNA Synthesis Kit (Bio-Rad). We performed all methods according to manufacturers' protocols.

To identify infection of 28 pathogens in wolves and raccoon dogs from inside and outside of the CEZ, we used a qPCR approach. We then sent cDNA to IDEXX Laboratories for quantitative PCR analyses (for samples list see Table 3.1). Subsequent analyses were conducted

at IDEXX Laboratories (Sacramento, CA) and included IDEXX Canine Fever of Unknown Origin RealPCR™ Panel (Comprehensive) and Canine Respiratory Disease (CRD) RealPCR™ Panel (Comprehensive) testing panels. We used a Two Proportions Z-tests to assess significant differences in prevalence between the CEZ and Northern Belarus and a Mann-Whitney-U test to assess significant associations between parasite infection and radiation exposure.

Blood-borne Parasite Transcriptome Identification

From a separate study examining gene expression (Chapter 2) we had RNAseq data. We took advantage of these data to identify herpes viral infection in wolves and raccoon dogs inside and outside of the Chernobyl Exclusion Zone (Table 3.1). Detailed methods for RNAseq are provided in chapter 2. Briefly, we extracted RNA from whole blood from each individual, prepared Illumina sequencing libraries using 1µg RNA, and sequenced all samples on two Illumina NextSeq PE75 High Output Flow Cells (Georgia Genomics and Bioinformatics Core, University of Georgia). We filtered raw reads with rCorrector (Song & Florea, 2015) and imported raw reads into Taxonomer (Uphoff et al. 2019) to screen for herpesvirus matches. We then compared Herpesviridea counts between individuals from the CEZ and northern Belarus using ANOVA and associations of herpesviridea intensity and internal Cs-137 radioactivity using general linear models.

RESULTS

Gastrointestinal Parasites

For gastrointestinal parasites we examined richness, prevalence, and egg and oocyte burdens in hosts and then made comparisons across species and levels of radiation exposure. We identified helminth and protozoan gastrointestinal parasites from 10 different genera in wolves (8 helminth and 2 protozoan) and from 5 genera in raccoon dogs (4 helminth and 1 protozoan)

(Table 3.2). Of these parasites identified, three groups (*Coccidia* ssp, *Toxocara* spp, and *Trichuris* spp.) can be directly transmitted, while the others are indirectly transmitted. Within any single fecal sample we observed between zero and six or zero and four different genera in wolf and raccoon dog samples respectively. Overall prevalence of hosts infected by at least one gastrointestinal parasite did not differ (Fisher's chi-square test: $p = 0.4905$) between host species. Additionally, co-infection rates did not differ between wolves and raccoon dogs (Fisher's chi-square test: $p = 1.0$). Prevalence of the three most common gastrointestinal parasites showed some differences between host species, though only differences in *Trichuris* species counts were significantly different (*Coccidia* spp. ($p = 0.812$), *Trichuris* spp. ($p = 0.001$), and *Alaria spp.* ($p = 0.623$), Figure 1A).

To begin to address the potential for radiation to play a role in parasite infection, we analyzed parasite richness with relation to radiation exposure, and found variation in parasite richness was not related to estimated radiation exposure levels for either host species (wolf: $p = 0.37$; raccoon dog: $p = 0.565$). We did see a trend of higher parasite prevalence in high radiation zones in all four of the most common gastrointestinal parasite groups for wolves (Figure 1A). However, none of these trends were significant (GLM: *Alaria* spp, adjusted $R^2 = -0.03088$, $p = 0.6107$, *Coccidia* spp adjusted $R^2 = -0.0205$, $p = 0.479$, *Trichuris* spp adjusted $R^2 = 0.01523$, $p = 0.236$, *Toxocara* spp. adjusted $R^2 = -0.02718$, $p = 0.501$). Parasite prevalence trends differed in raccoon dogs, with *Coccidia* showing a trend of higher prevalence in low radiation areas and *Tirchuris* spp. and *Alraia* spp. showing a trend of higher prevalence in high radiation zones. None of these trends were significant (*Alaria* spp $p = 0.8229$, *Coccidia* spp $p = 0.311$, *Trichuris* spp $p = 0.2679$). (Figure 1A)

Gastrointestinal parasite burdens did not significantly differ among fecal samples collected in low and high radiation areas, even in the most common parasites. With no difference in *Alaria* spp. oocytes in wolves (Mann-Whitney *U*-test: F(1,32) = 0.004, p = 0.947) or raccoon dogs (Mann-Whitney *U*-test: F(1,15) = 0.589, p = 0.455) (Figure 1B), or *Trichuris* spp. eggs for both host species, (Figure 1C, Mann-Whitney *U*-test: wolves F(1,30) = 1.252, p= 0.272, raccoon dogs F(1,2) = 0.013, p= 0.754).

Serological Testing

For a subset of animals that we trapped within the CEZ we were able to perform serological testing to estimate exposure rates for common canid microparasites. Based on these analyses we detected antibodies for *Borrelia burgdorferi* and *Dirofilaria immitis,* two indirectly transmitted blood-borne parasties. as well as a directly transmitted pathogen, Canine parvovirus (Table 3.3). Within the wolves, antibodies to all three of these pathogens were found, however only one wolf tested positive for more than one. Within raccoon dogs we only found evidence of antibody production against *D. immitis*. The overall low apparent exposure rates preclude much comparison across hosts with different radiation body burdens. However, 57.14% of wolves were seropositive for Canine parvovirus and these individuals had significantly higher body burdens than those testing negative (Welch's t-test: $t = 3.916$, df = 2.309, $p = 0.047$).

Blood-borne Parasite qPCR Identification

In addition to the field-based rapid serological testing on a subset of animals, we were able to test a larger number of hosts from both the CEZ and northern Belarus across a wider panel of blood-borne parasites with a qPCR approach. Of the 28 blood-borne parasites tested (Figure B1), we found evidence of only seven parasites across all host samples (Table 3.4). The most prevalent blood-borne parasites identified were two indirectly transmitted parasites,

Babesia and *Hepatozoon* species. *Babesia* species were characterized in both raccoon dogs and wolves, but only in individuals from the CEZ. Whereas *Hepatozoon* species were only characterized in wolves, and in individuals from both the CEZ and northern Belarus. Coinfection was not common in our samples. Transmission strategies of the parasites identified varied, with four indirectly transmitted parasites (*Anaplasma*, *Babesia*, *Hepatozoan* and *Richettsia* spp) and three directly transmitted parasites (Canine distemper virus, Coronavirus, and *Streptococcus equi zooepidemicus*). Surprisingly, all but one blood-borne parasite, *Hepatozoon* sp., were found exclusively in individuals from either the CEZ or northern Belarus. Prevalence patterns were low across our tested individuals, not allowing an in depth analysis of individual radiation exposure and blood-parasite infection patterns.

Blood-borne Parasites Transcriptome Identification

Blood transcriptome sequence screening resulted in successful identification of Herpesviridae viral sequences in all individuals with the exception of one wolf from northern Belarus. Transcriptome results showed differences (ANOVA: $p = 0.026$) in viral intensity between wolves from the CEZ (mean = 58.22 ± 44.77) and northern Belarus (mean = 12.66 ± 10.56), and herpesvirus read counts were associated with wolf radiation body burden (GLM: adjusted $R^2 = 0.6482$, $df = 0.403$, $p = 0.005$, Figure 2) in individuals from the CEZ. Conversely, Taxonomer results showed no difference (ANOVA: $p = 0.964$) in viral intensity between raccoon dogs from the CEZ (mean = 78.5 ± 26.14) and northern Belarus (mean = 77.5 ± 24.74), and no association with radiation exposure estimates (GLM: adjusted $R^2 = -0.1926$, $df = 0.026$, $p = 0.6835$).

DISCUSSION

Our study represents the first assessment of macro- and microparasite communities across two sympatric canid species in the CEZ. In addition, it is the first to describe a diversity of parasites in canids from the region while reporting parasite prevalence with individual estimates ionizing radiation exposure, when appropriate. Overall, our results suggest the parasite communities in canids from the CEZ are comprised primarily of generalist parasites, of which, most have indirect life cycles, requiring intermediate hosts to complete their life cycle. Additionally, many of these parasites have life cycles with several transmission strategies. In total, we found evidence of 18 and 9 parasite infections in wolves and raccoon dogs, respectively. Of the parasite infections identified, we found variation across host species, population, radiation exposure, and parasite species.

Studies suggest that radiation exposure may negatively impact gastrointestinal parasites through reduced reproduction and survival (Oothuman et al., 1978; Pleass & Bianco, 1995). The gastrointestinal parasite infection patterns observed here did not differ across radiation exposures within the CEZ. The potential for immunocompromising effects of radiation exposure (Camplani et al., 1999) on hosts, combined with reduced survival and reproduction described in some gastrointestinal parasites as a result of radiation exposure (Chai et al., 1995), may both contribute to the lack of a difference in gastrointestinal infections across radiation exposures.

Additionally, we described gastrointestinal parasites prevalence patterns across host species. Prevalence of *Trichuris* spp was higher in wolves than raccoon dogs, however *Trichuris* prevalence in wolves was still higher than described decades prior (Möhl et al., 2009). While raccoon dogs have been documented to exhibit infection with *Toxocara* spp. infection (Duscher et al., 2017) we only identified *Toxocara* spp. in wolves in the CEZ. Previous studies of wolves

in southern Belarus documented 22% prevalence of *Toxocara* spp. (Möhl et al., 2009), which is similar to the prevalence we found in the low radiation regions. Additionally, *Alaria* spp. intensity was higher in wolves from the CEZ and at higher prevalence than described previously in southern Belarus (Shimalov & Shimalov, 2000). Interestingly, *Alaria spp.* intensity was lower than expected in raccoon dogs, despite raccoon dogs being highly susceptible to *Alaria alata* infections (Al-Sabi et al., 2013; Laurimaa et al., 2016). *Alaria* transmission involves two intermediate hosts, snails and tadpoles (Möhl et al., 2009) and definitive host infection occurs by ingesting tadpoles or other infected paratenic hosts. Raccoon dogs more commonly eat amphibians, suggesting they might show higher oocyte intensities (Kauhala & Kowalczyk, 2011). However, *Alaria* mesocercariae may bioaccumulate in other paratenic hosts and facilitate transmission to wolves through trophic transfer (Möhl et al., 2009). Raccoon dogs were also the only host species to exhibit *Moniezia spp.,* and while *Moniezia spp.* infection patterns are not well understood in wolves or raccoon dogs from the region, wolves are known to have low (0.5%) prevalence in Canada (Stronen, Sallows, Forbes, Wagner, & Paquet, 2011).

Serological testing showed similar differences of parasite infections across wolves and raccoon dogs. A high proportion of wolves were seropositive for CPV (57.14%), however this does not differ from studies in other wolf populations (Johnson, Boyd, & Pletscher, 1994; Mech & Goyal, 2011). Interestingly, 12.5% of wolves tested seropositive for heartworm, *Dirofilaria immitis*, while two of the three raccoon dogs (66.7%) tested were seropositive. This rate is far higher in raccoon dogs than the 7% described in individuals from Japan (Kido et al., 2011). However, given our small sample size of raccoon dogs this may not be a reflection of population infection rates. Wolf *Dirofilaria immitis* patterns across Europe are not well described, however infection has been identified in other regions (Pascucci et al., 2007; Penezić, Selaković, Pavlović,

& Ćirović, 2014; Shimalov & Penkevich, 2012). One might expect low rates of infection with *D. immitis* in the CEZ because mosquito vectors are known to be detrimentally impacted by gamma radiation exposure, with altered life history traits such as fecundity, hatchability, adult emergence, sex ratio and longevity at doses of 3-50 Gy (Shetty et al., 2016). However, changes in canid behavior in the CEZ could counteract impacts on mosquitos. More research is needed to asses these patterns within the CEZ.

Blood-borne parasite prevalence in our study was distinct between host species. *Babesia* spp. were the only microparasite infecting both wolves and raccoon dogs. Canine distemper virus and coronavirus, two directly transmitted microparasites, were only identified in wolves from Northern Belarus. While tick born microparasites (*Babesia canis canids* and/or *Babesia conradae, Rickettsia* spp., *Hepatozoon canis, Streptococcus equi zooepidemicus*) were most commonly identified in raccoon dogs from the CEZ. This pattern of tick born microparasites is not surprising as tick densities (Efremova et al., 2017) and questing ticks with confirmed microparasite infection with *Rickettsia raoultii*, *Bartonella spp*. (Rogovskyy et al., 2019) and *Anaplasma phagoctophilum* have been found at higher prevalence in the CEZ than surrounding farmland. However, the differences between host infection patterns suggest potential differences in infection dynamics and susceptibility. Interestingly, only *Hepatozoon canis* occurred in individuals from both locations and all other microparasites were found in one population or the other.

Many host-viral dynamics show a non-linear response to radiation exposure (Lee 2012, Hume 2016). High doses can render viruses inactive (Lee et al. 2012, Hume et al. 2016), while low doses (2.5-25 Gy) can activate replication in viruses such as herpes simplex virus (Mezhir et al 2005) and parvoviruses (Walz et al. 1992, Alexander et al. 1996). Our study describes a high

proportion (57.14%) of wolves tested seropositive for Canine parvovirus (CPV) in the CEZ. This is a higher prevalence than what has been seen in other parts of Europe (e.g. 12-15% in France (Molnar et al., 2014). Additionally, wolves which were seropositive for CPV had significantly higher Cs-137 body burdens. This is concerning given that CPV is a highly contagious pathogen (Nandi and Kumar 2010) associated with high wolf morbidity and mortality (Johnson et al. 1994; Pence 1995; Gese et al. 1997; DiSabatino et al. 2014). CPV severity and virulence can be influenced by specific genetic and antigenic mutations, as found in CPV strains found across Europe (Miranda & Thompson, 2016), and radiation induced mutations could affect CPV virulence. New research from the CEZ identified two new adeno-associated viruses in bank voles (Mezhir et al. 2019), yet the prevalence and infection load of these strains was not associated with environmental radiation exposure. Given the high CPV seroprevalence, further examination of mutations of CPV within the CEZ are warranted.

Additionally, our study found increased herpesvirus transcription in wolves from the CEZ and transcript intensity correlated with radiation body burden. European wolf herpesvirus infection rates are not well described, however seroprevalence is generally high (87%) in some north American populations (Almberg et al., 2009). Yet, canine herpesvirus causes mortality in neonates (Carmichael, 1965) and immunosuppressed hosts (Malone et al., 2001), and can affect reproductive success (Morresey, 2004). Herpesviridae infections can additionally interact with other host genomic characteristics and retroviruses to develop more serious diseases (Chen et al., 2019; Morahan et al., 1989).

Interestingly raccoon dogs did not show similar patterns of herpesvirus transcript abundances between the CEZ and northern Belarus, nor did their transcript abundances correlate with body burden. This may be a true artifact of differential sensitivity to herpesvirus infection or

activation. However, little research has been conducted on raccoon dog herpesvirus infection patterns. Low sample sizes in northern Belarus may additionally decrease the power of our comparisons of herpesvirus activation between these two sampling locations. The raccoon dog radiation exposure estimates were also calculated from soil area weighted means from the trap location of each individual. These body burden estimates are not as exact as internal radioactivity readings provided for the wolves and may also have played a role in our findings. Nevertheless, raccoon dogs did exhibit higher overall herpesvirus transcription than wolves.

While our findings describe complex patterns of infection, differing between hosts and parasites, our estimates are general under representations of true parasite communities in canids from the region. By using qPCR, serological, and coprological rather than necropsy techniques (Torres et al 2001) and freezing fecal (e.g. Bowman 2009) samples we diminished our ability for identification of some parasites. To better assess epidemiological factors shaping the parasite communities infecting these free-ranging canids, further investigations of sympatric and preferred prey species should be examined.

Additionally, the CEZ is a protected area where human activity has been significantly limited following the reactor explosion in 1986 and implementation of the CEZ, and diverse and abundant mammal communities are now characterized in this area (Deryabina et al. 2015, Webster et al. 2016, Schlichting et al. 2019). Like many regions in the western world, wolves and raccoon dogs are persecuted in Belarus, with a reward for each individual removed from the population. Canids avoid regions where they are more likely to encounter negative human interaction (Tigas et al., 2002), and thus may be avoiding the CEZ edge which borders agricultural land. Instead, canids may preferentially use the center of the CEZ which lacks human activity but is more highly contaminated. This lack of human activity in some of the most

highly contaminated regions of the CEZ may result in higher canid densities, and thus increased transmission rates between conspecific hosts. Of the parasites we examined, many are likely to experience density dependent transmission. Our study has provided baseline data on parasite communities in canids of this region, but additional studies are needed to disentangle the numerous factors contributing to transmission and prevalence. In particular, our finding of increased herpesviruses in wolves, as a function of Cs-137 body burden, could have serious impacts on reproduction and thus population dynamics and requires further study.

REFERENCES

Acevedo-Whitehouse, K., & Duffus, A. L. J. (2009). Effects of environmental change on wildlife health. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1534), 3429–3438. https://doi.org/10.1098/rstb.2009.0128

Adamovich, V. L. (1998). Rabies in Animals Inhabiting Radioactively Contaminated Territories. *Russian Journal of Ecology*, *3*(29), 205–208.

Agate, L., Mariotti, S., Elisei, R., Mossa, P., Pacini, F., Molinaro, E., … Pinchera, A. (2008). Thyroid autoantibodies and thyroid function in subjects exposed to chernobyl fallout during childhood: Evidence for a transient radiation-induced elevation of serum thyroid antibodies without an increase in thyroid autoimmune disease. *Journal of Clinical Endocrinology and Metabolism*, *93*(7), 2729–2736. https://doi.org/10.1210/jc.2008-0060

Al-Sabi, M. N. S., Chriél, M., Jensen, T. H., & Enemark, H. L. (2013). Endoparasites of the raccoon dog (Nyctereutes procyonoides) and the red fox (Vulpes vulpes) in Denmark 2009-2012 - A comparative study. *International Journal for Parasitology: Parasites and Wildlife*, *2*(1), 144–151. https://doi.org/10.1016/j.ijppaw.2013.04.001

Almberg, E. S., Mech, L. D., Smith, D. W., Sheldon, J. W., & Crabtree, R. L. (2009). A serological survey of infectious disease in yellowstone national park's canid community. *PLoS ONE*, *4*(9). https://doi.org/10.1371/journal.pone.0007042

Bichet, C., Scheifler, R., Cœurdassier, M., Julliard, R., Sorci, G., & Loiseau, C. (2013). Urbanization, trace metal pollution, and malaria prevalence in the house sparrow. *PloS One*, *8*(1), e53866. https://doi.org/10.1371/journal.pone.0053866

Bonisoli-Alquati, A., Mousseau, T. a, Møller, A. P., Caprioli, M., & Saino, N. (2010). Increased oxidative stress in barn swallows from the Chernobyl region. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *155*(2), 205–210. https://doi.org/10.1016/j.cbpa.2009.10.041

Bonzom, J. M., Hättenschwiler, S., Lecomte-Pradines, C., Chauvet, E., Gaschak, S., Beaugelin-Seiller, K., … Adam-Guillermin, C. (2016). Effects of radionuclide contamination on leaf litter decomposition in the Chernobyl exclusion zone. *Science of the Total Environment*, *562*, 596–603. https://doi.org/10.1016/j.scitotenv.2016.04.006

Booth, G. H. J., & Schulert, A. R. (1968). Zinc metabolism in Schistosomes. *Proceeding of Society of Experimental Biology and Medicine*, *127*(3), 700–704. https://doi.org/10.3181/00379727-127-32777

Buck, J. C., Hua, J., Brogan, W. R., Dang, T. D., Urbina, J., Bendis, R. J., … Relyea, R. a. (2015). Effects of Pesticide Mixtures on Host-Pathogen Dynamics of the Amphibian Chytrid Fungus. *Plos One*, *10*(7), e0132832. https://doi.org/10.1371/journal.pone.0132832

Camplani, A., Saino, N., & Møller, A. P. (1999). Carotenoids, sexual signals and immune function in barn swallows from Chernobyl. *Proceedings of the Royal Society B: Biological Sciences*, *266*(1424), 1111–1116. https://doi.org/10.1098/rspb.1999.0751

Carmichael, L. (1965). Clinical and pathologic features of a fatal viral disease of newborn pups. *American Journal of Veterinary Research*, *26*(113), 803.

Chai, J., Kim, S., Kook, J., & Lee, S. (1995). Effects of gamma-irradiation on survival and development of metageonimus yokogawai metacercariae in rats. *The Korean Journal of Parasitology*, *33*(4), 297–303.

Chen, J., Foroozesh, M., & Qin, Z. (2019). Transactivation of human endogenous retroviruses by tumor viruses and their functions in virus-associated malignancies. *Oncogenesis*, *8*(1). https://doi.org/10.1038/s41389-018-0114-y

Dainiak, N., Waselenko, J. K., Armitage, J. O., MacVittie, T. J., & Farese, A. M. (2003). The hematologist and radiation casualties. *Hematology / the Education Program of the American Society of Hematology. American Society of Hematology. Education Program*, 473–496. https://doi.org/10.1182/asheducation-2003.1.473

Dharmarajan, G., Beasley, J. C., Fike, J. a., & Rhodes, O. E. (2009). Population genetic structure of raccoons (Procyon lotor) inhabiting a highly fragmented landscape. *Canadian Journal of Zoology*, *87*(9), 814–824. https://doi.org/10.1139/Z09-072

Duscher, T., Hod, A., Glawischnig, W., & Duscher, G. G. (2017). *The raccoon dog (Nyctereutes procyonoides) and the raccoon (Procyon lotor) — their role and impact of maintaining and transmitting zoonotic diseases in Austria , Central Europe*. 1411–1416. https://doi.org/10.1007/s00436-017-5405-2

Efremova, G. A., Bychkova, E. I., Movila, A. A., & Jakovich, M. M. (2017). *TICKS (ACARI : IXODIDAE) IN PSRER : ECOLOGY , GENETIC POLYMORPHISM AND EPIDEMIOLOGICAL - EPIZOOTIC SIGNIFICANCE*. 1–6.

Eisenbud, M., & Gesell, T. F. (1997). *Environmental Radioactivity from Natural, Industrial and*

Military Sources: From Natural, Industrial and Military Sources. Elsevier.

Gese, E. M. (1998). Survey and census techniques for hyaenas. *Hyaenas. Status Survey and Conservation Action Plan*, (June), 88–91.

Gridley, D. S., Pecaut, M. J., Miller, G. M., Moyers, M. F., & Nelson, G. A. (2001). Dose and dose rate effects of whole-body γ-Irradiation: II. hematological variables and cytokines. *In Vivo*, *15*(3), 209–216.

Izrael, Y., & Bogdevich, I. (2009). *Atlas of current and predicted consequences of the Chernobyl accident on the affected territories of Russia and Belarus*. Minsk, Belarus: Belkartographia.

Johnson, M. R., Boyd, D. K., & Pletscher, D. H. (1994). Serologic investigations of canine parvovirus and canine distemper in relation to wolf (Canis lupus) pup mortalities. *Journal of Wildlife Diseases*, *30*(2), 270–273. https://doi.org/10.7589/0090-3558-30.2.270

Kauhala, K., & Kowalczyk, R. (2011). Invasion of the raccoon dog Nyctereutes procyonoides in Europe : History of colonization , features behind its success , and threats to native fauna. *Current Zoology*, *57*(5), 584–598.

Kesäniemi, J., Lavrinienko, A., Tukalenko, E., Mappes, T., Watts, P. C., & Jurvansuu, J. (2019). Infection load and prevalence of novel viruses identified from the bank vole do not associate with exposure to environmental radioactivity. *Viruses*, *12*(1). https://doi.org/10.3390/v12010044

Kennamer, R. A., Oldenkamp, R. E., Leaphart, J. C., King, J. D., Bryan, A. L., & Beasley, J. C. (2017). Radiocesium in migratory aquatic game birds using contaminated U.S. Department of Energy reactor-cooling reservoirs: A long-term perspective. *Journal of Environmental Radioactivity*, *171*, 189–199. https://doi.org/10.1016/j.jenvrad.2017.02.022

Kido, N., Wada, Y., Takahashi, M., Kamegaya, C., Omiya, T., & Yamamoto, Y. (2011). Prevalence of Dirofilaria immitis infection in living raccoon dogs assessed by hematological examination. *Journal of Veterinary Medical Science*, *73*(6), 845–847. https://doi.org/10.1292/jvms.10-0512

King, K. C., Daniel Mclaughlin, J., Boily, M., & Marcogliese, D. J. (2010). Effects of agricultural landscape and pesticides on parasitism in native bullfrogs. *Biological Conservation*, *143*(2), 302–310. https://doi.org/10.1016/j.biocon.2009.10.011

Laurimaa, L., Süld, K., Davison, J., Moks, E., Valdmann, H., & Saarma, U. (2016). Alien species and their zoonotic parasites in native and introduced ranges: The raccoon dog example. *Veterinary Parasitology*, *219*, 24–33. https://doi.org/10.1016/j.vetpar.2016.01.020

Liu, S.-Z. (2003). Nonlinear dose-response relationship in the immune system following exposure to ionizing radiation: mechanisms and implications. *Nonlinearity in Biology, Toxicology, Medicine*, *1*(1), 71–92.

Lourenço, J., Mendo, S., & Pereira, R. (2016). Radioactively contaminated areas: Bioindicator species and biomarkers of effect in an early warning scheme for a preliminary risk assessment. *Journal of Hazardous Materials*, *317*, 503–542. https://doi.org/10.1016/j.jhazmat.2016.06.020

Lourenço, J., Pereira, R., Gonçalves, F., & Mendo, S. (2013). Metal bioaccumulation, genotoxicity and gene expression in the European wood mouse (Apodemus sylvaticus) inhabiting an abandoned uranium mining area. *Science of the Total Environment*, *443*, 673–680. https://doi.org/10.1016/j.scitotenv.2012.10.105

Love, C. N., Winzeler, M. E., Beasley, R., Scott, D. E., Nunziata, S. O., & Lance, S. L. (2016). Patterns of Amphibian infection prevalence across wetlands on the Savannah River Site,

South Carolina, USA. *Diseases of Aquatic Organisms*, *121*(1), 1–14. https://doi.org/10.3354/dao03039

Malone, E. K., Ledbetter, E. C., Rassnick, K. M., Kim, S. G., & Russell, D. (2001). Case report : Case report. *Canadian Family Physician*, *47*(10), 788–789.

Manda, K., Glasow, A., Paape, D., & Hildebrandt, G. (2012). Effects of ionizing radiation on the immune system with special emphasis on the interaction of dendritic and T cells. *Frontiers in Oncology*, *2*(August), 1–9. https://doi.org/10.3389/fonc.2012.00102

Marcogliese, D. J., Brambilla, L. G., Gagné, F., & Gendron, A. D. (2005). Joint effects of parasitism and pollution on oxidative stress biomarkers in yellow perch Perca flavescens. *Diseases of Aquatic Organisms*, *63*(1), 77–84. https://doi.org/10.3354/dao063077

Marcogliese, D. J., & Pietrock, M. (2011). Combined effects of parasites and contaminants on animal health: parasites do matter. *Trends in Parasitology*, *27*(3), 123–130. https://doi.org/10.1016/j.pt.2010.11.002

Martin, K. (2001). Wildlife in alpine and sub-alpine habitats. *Wildlife Habitats and Relationships in Oregon And*, 285–310. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Wildlife+in+Alpine+and+Sub-alpine+Habitats#0

Mech, L. D., & Goyal, S. M. (2011). Parsing demographic effects of canine parvovirus on a Minnesota wolf population. *Journal of Veterinary Medicine and Animal Health*, *3*(2), 27–30. Retrieved from http://www.academicjournals.org/jvmah/PDF/2011/June/Mech and Goyal.pdf

Miranda, C., & Thompson, G. (2016). Canine parvovirus: The worldwide occurrence of antigenic variants. *Journal of General Virology*, *97*(9), 2043–2057.

https://doi.org/10.1099/jgv.0.000540

Möhl, K., Große, K., Hamedy, A., Wüste, T., Kabelitz, P., & Lücker, E. (2009). Biology of Alaria spp. and human exposition risk to Alaria mesocercariae-a review. *Parasitology Research*, *105*(1), 1–15. https://doi.org/10.1007/s00436-009-1444-7

Møller, A. P., Hobson, K. A., Mousseau, T. A., & Peklo, A. M. (2006). Chernobyl as a population sink for barn swallows: Tracking dispersal using stable-isotope profiles. *Ecological Applications*, *16*(5), 1696–1705. https://doi.org/10.1890/1051-0761(2006)016[1696:CAAPSF]2.0.CO;2

Møller, A. P., & Mousseau, T. A. (2013). Assessing effects of radiation on abundance of mammals and predator-prey interactions in Chernobyl using tracks in the snow. *Ecological Indicators*, *26*, 112–116. https://doi.org/10.1016/j.ecolind.2012.10.025

Møller, A. P., Bonisoli-Alquati, A., Rudolfsen, G., & Mousseau, T. A. (2011). Chernobyl birds have smaller brains. *PLoS ONE*, *6*(2). https://doi.org/10.1371/journal.pone.0016862

Møller, A. P., & Mousseau, T. a. (2009). Reduced abundance of insects and spiders linked to radiation at Chernobyl 20 years after the accident. *Biology Letters*, *5*(3), 356–359. https://doi.org/10.1098/rsbl.2008.0778

Molnar, B., Duchamp, C., Möstl, K., Diehl, P. A., & Betschart, B. (2014). Comparative survey of canine parvovirus, canine distemper virus and canine enteric coronavirus infection in free-ranging wolves of central Italy and south-eastern France. *European Journal of Wildlife Research*, *60*, 613–624. https://doi.org/10.1007/s10344-014-0825-0

Morahan, P. S., Mama, S., Anaraki, F., & Leary, K. (1989). Molecular localization of abortive infection of resident peritoneal macrophages by herpes simplex virus type 1. *Journal of Virology*, *63*(5), 2300–2307. https://doi.org/10.1128/jvi.63.5.2300-2307.1989

Morley, N. J. (2012). The effects of radioactive pollution on the dynamics of infectious diseases in wildlife. *Journal of Environmental Radioactivity*, *106*, 81–97. https://doi.org/10.1016/j.jenvrad.2011.12.019

Morley, N. J., Lewis, J. W., & Hoole, D. (2006). Pollutant-induced effects on immunological and physiological interactions in aquatic host–trematode systems: implications for parasite transmission. *Journal of Helminthology*, *80*(2), 137–149. https://doi.org/10.1079/joh2006345

Morresey, P. R. (2004). Reproductive effects of canine herpesvirus. *Compendium on Continuing Education for the Practicing Veterinarian*, *26*(10), 804–811.

Mothersill, C., Bucking, C., Smith, R. W., Agnihotri, N., O'Neill, A., Kilemade, M., & Seymour, C. B. (2006). Communication of radiation-induced stress or bystander signals between fish in vivo. *Environmental Science and Technology*, *40*(21), 6859–6864. https://doi.org/10.1021/es061099y

Mousseau, T. A., Milinevsky, G., Kenney-Hunt, J., & Møller, A. P. (2014). Highly reduced mass loss rates and increased litter layer in radioactively contaminated areas. *Oecologia*, *175*(1), 429–437. https://doi.org/10.1007/s00442-014-2908-8

Mousseau, T. A., Welch, S. M., Chizhevsky, I., Bondarenko, O., Milinevsky, G., Tedeschi, D. J., … Møller, A. P. (2013). Tree rings reveal extent of exposure to ionizing radiation in Scots pine Pinus sylvestris. *Trees - Structure and Function*, *27*(5), 1443–1453. https://doi.org/10.1007/s00468-013-0891-z

Nansen, P., Christensen, N., & Frandsen, F. (1976). A technique for in vivo labelling of Fascioa hepatica miracida with radioselenium. *Zeitschrift Fur Parasitenkunde*, *49*(1), 73–78.

Oothuman, P., Denham, D. A., McGreevy, P. B., & Nelson, G. S. (1978). Studies With Brugia

Pahangi. 15. Cobalt 60 Irradiation of the Worm. *Journal of Helminthology*, *52*(2).

Pascucci, I., Fico, R., Angelo, A. R. D., Serini, S., & Cammà, C. (2007). *Prima segnalazione in Italia di filariosi cardiopolmonare nel lupo (Canis lupus)*. *43*(4), 843–850.

Penezić, A., Selaković, S., Pavlović, I., & Ćirović, D. (2014). First findings and prevalence of adult heartworms (Dirofilaria immitis) in wild carnivores from Serbia. *Parasitology Research*, *113*(9), 3281–3285. https://doi.org/10.1007/s00436-014-3991-9

Pleass, R. J., & Bianco, A. E. (1995). The effects of gamma radiation on the development of Heligmosomoides polygyrus bakeri in mice. *International Journal for Parasitology*, *25*(9), 1099–1109. https://doi.org/10.1016/0020-7519(95)00010-Y

Rhodes, O. E., Bréchignac, F., Bradshaw, C., Hinton, T. G., Mothersill, C., Arnone, J. A., … Zimmerman, J. K. (2020). Integration of ecosystem science into radioecology : A consensus perspective. *Science of the Total Environment*, *740*, 140031. https://doi.org/10.1016/j.scitotenv.2020.140031

Rogovskyy, A. S., Threadgill, D. W., Akimov, I. A., Nebogatkin, I. V., Rogovska, Y. V., Melnyk, M. V., & Rogovskyy, S. P. (2019). Borrelia and Other Zoonotic Pathogens in Ixodes ricinus and Dermacentor reticulatus Ticks Collected from the Chernobyl Exclusion Zone on the 30th Anniversary of the Nuclear Disaster. *Vector-Borne and Zoonotic Diseases*, *19*(7), 466–473. https://doi.org/10.1089/vbz.2018.2318

Ruiz-Rodriguez, M., Møller, A. P., Mousseau, T. A., & Soler, J. J. (2017). Capacity of blood plasma is higher in birds breeding in radioactively contaminated areas. *PLoS ONE*, *12*(6), 1–12. https://doi.org/10.1371/journal.pone.0179209

Sazykina, T., & Kryshev, I. I. (2006). Radiation effects in wild terrestrial vertebrates - the EPIC collection. *Journal of Environmental Radioactivity*, *88*(1), 11–48.

https://doi.org/10.1016/j.jenvrad.2005.12.009

Schlichting, P. E., Love, C. N., Webster, S. C., & Beasley, J. C. (2019). Efficiency and composition of vertebrate scavengers at the land-water interface in the Chernobyl Exclusion Zone. *Food Webs*, *18*. https://doi.org/10.1016/j.fooweb.2018.e00107

Schröder, S., Kriesen, S., Paape, D., Hildebrandt, G., & Manda, K. (2018). Modulation of inflammatory reactions by low-dose ionizing radiation: Cytokine release of murine endothelial cells is dependent on culture conditions. *Journal of Immunology Research*, *2018*. https://doi.org/10.1155/2018/2856518

Shetty, V., Shetty, N. J., Harini, B. P., Ananthanarayana, S. R., Jha, S. K., & Chaubey, R. C. (2016). Effect of gamma radiation on life history traits of Aedes aegypti (L.). *Parasite Epidemiology and Control*, *1*(2), 26–35. https://doi.org/10.1016/j.parepi.2016.02.007

Shimalov, V. V., & Penkevich, V. A. (2012). Helminth Fauna of the wolf (Canis lupus linnaeus, 1758) in Belorussian Polesie. *Parazitologiya*, Vol. 46, pp. 118–126.

Shimalov, V. V, & Shimalov, V. T. (2000). Helminth fauna of the wolf (Canis lupus Linnaeus , 1758) in Belorussian Polesie. *Parastiology Research*, *86*, 163–164.

Song, L., & Florea, L. (2015). Rcorrector: Efficient and accurate error correction for Illumina RNA-seq reads. *GigaScience*, *4*(1), 1–8. https://doi.org/10.1186/s13742-015-0089-y

Stronen, A. V., Sallows, T., Forbes, G. J., Wagner, B., & Paquet, P. C. (2011). Diseases and parasites in wolves of the riding mountain national park region, manitoba, canada. *Journal of Wildlife Diseases*, *47*(1), 222–227. https://doi.org/10.7589/0090-3558-47.1.222

Thielen, F., Zimmermann, S., Baska, F., Taraschewski, H., & Sures, B. (2004). *The intestinal parasite Pomphorhynchus laevis (Acanthocephala) from barbel as a bioindicator for metal pollution in the Danube River near Budapest , Hungary*. *129*, 421–429.

https://doi.org/10.1016/j.envpol.2003.11.011

Tigas, L. A., Van Vuren, D. H., & Sauvajot, R. M. (2002). Behavioral responses of bobcats and coyotes to habitat fragmentation and corridors in an urban environment. *Biological Conservation*, *108*(3), 299–306. https://doi.org/10.1016/S0006-3207(02)00120-9

Vieira, H. G. S., Grynberg, P., Bitar, M., Da Fonseca Pires, S., Hilário, H. O., Macedo, A. M., … Franco, G. R. (2014). Proteomic analysis of Trypanosoma cruzi response to ionizing radiation stress. *PLoS ONE*, *9*(5). https://doi.org/10.1371/journal.pone.0097526

Wallace, R. B., Daniels, C. E., & Altman, J. (1972). Behavioral effects of neonatal irradiation of the cerebellum. III. Qualitative observations in aged rats. *Developmental Psychobiology*, *5*(1), 35–41. https://doi.org/10.1002/dev.420050105

Webster, S. C., Byrne, M. E., Lance, S. L., Love, C. N., Hinton, T. G., Shamovich, D., & Beasley, J. C. (2016). Where the wild things are: Influence of radiation on the distribution of four mammalian species within the Chernobyl Exclusion Zone. *Frontiers in Ecology and the Environment*, *14*(4). https://doi.org/10.1002/fee.1227

Zuther, Johnson, J. J., Haselkorn, R., McLeod, R., & Gornicki, P. (1999). Growth of Toxoplasma gondii is inhibited by aryloxyphenoxypropionate herbicides targeting acetyl-CoA carboxylase. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(23), 13387–13392. https://doi.org/10.1073/pnas.96.23.13387

TABLES

Table 3.1. Number of gray wolf and raccoon dog samples analyzed from inside the CEZ and northern Belarus for each of the five different analytical techniques.

	Fecal Float	**ELISA based Serological Testing**	**TiterCHEK CPV/CDV**	**qPCR Identification**	**Herpesvirus Transcriptome ID**
Gray Wolves					
CEZ	58	9	7	10	9
Northern Belarus	0	0	0	8	9
Raccoon Dogs					
CEZ	25	3	3	9	5
Northern Belarus	0	0	0	3	2

Table 3.2. Comparing prevalence of 11 parasite groups idenitified from fecal float analysis of wolf and raccoon dog samples from high (wolf = 28, raccoon dog = 13) and low (wolf = 25, raccoon dog = 12) radiation zones collected in 2014 in the Chernobyl Exclusion Zone.

Parasite	Gray wolves		Raccoon dogs	
	Low Rad Prevalence (%)	High Rad Prevalence (%)	Low Rad Prevalence (%)	High Rad Prevalence (%)
Alaria spp.	50.0	76	61.5	36
Coccidia	42.8	52	61.5	38.46
Diphyllobothrium spp.	14.28	8	0	0
Dipylidium	0	4	0	0
Moniezia spp.	0	0	23.07	13.38
Paragonimus spp.	25.0	20	0	0
Physaloptera spp.	14.28	16	23.07	30.77
Sarcocystis spp.	7.14	12	0	0
Taenia spp.	0	4	0	0
Toxocara spp.	39.28	40	0	0
Trichuris spp.	50	68	4	23.08

Table 3.3. Seropositive testing results for gray wolves (*Canis lupus*) and raccoon dogs (*Nyctereutes procyonoides*) from inside the CEZ, with number of individuals tested (*n*) and percent prevalence for each test.

	Gray Wolf		**Raccoon Dogs**	
Microparasite	***n***	**Prevalence (%)**	***n***	**Prevalence (%)**
Ehlichia canis and/or *E. ewingii*	9	0	3	0
Borrelia burgdorfi	9	12.5	3	0
Anaplasmosis phgocytophilum and/or *A. platys*	9	0	3	0
Dirofilaria immitis	9	12.5	3	66.7
Canine distemper virus	7	0		
Canine parvovirus	7	57.14		

Table 3.4. Results for qPCR detection of 7 microparasites from blood samples of wolves and raccoon dogs from northern Belarus and the CEZ. Numbers represent percent individuals testing positive of all wolves tested (N. Belarus, N = 9; CEZ, N = 10) and raccoon dogs (N. Belarus, N = 3; CEZ, N = 8) tested.

Microparasite	Gray Wolf		Raccoon Dog	
	N. Belarus	CEZ	N. Belarus	CEZ
Anaplasma phagoctophilum	0	0.1	0	0
Babesia canis canids and *B. conradae*	0	0.1	0	0.85
Canine distemper virus	0.25	0	0	0
Coronavirus	0.125	0	0	0
Hepatozoon canis	0.5	0.2	0	0
Rickettsia spp.	0	0	0	0.33
Streptococcus equi zooepidemicus	0	0	0	0.33

FIGURES

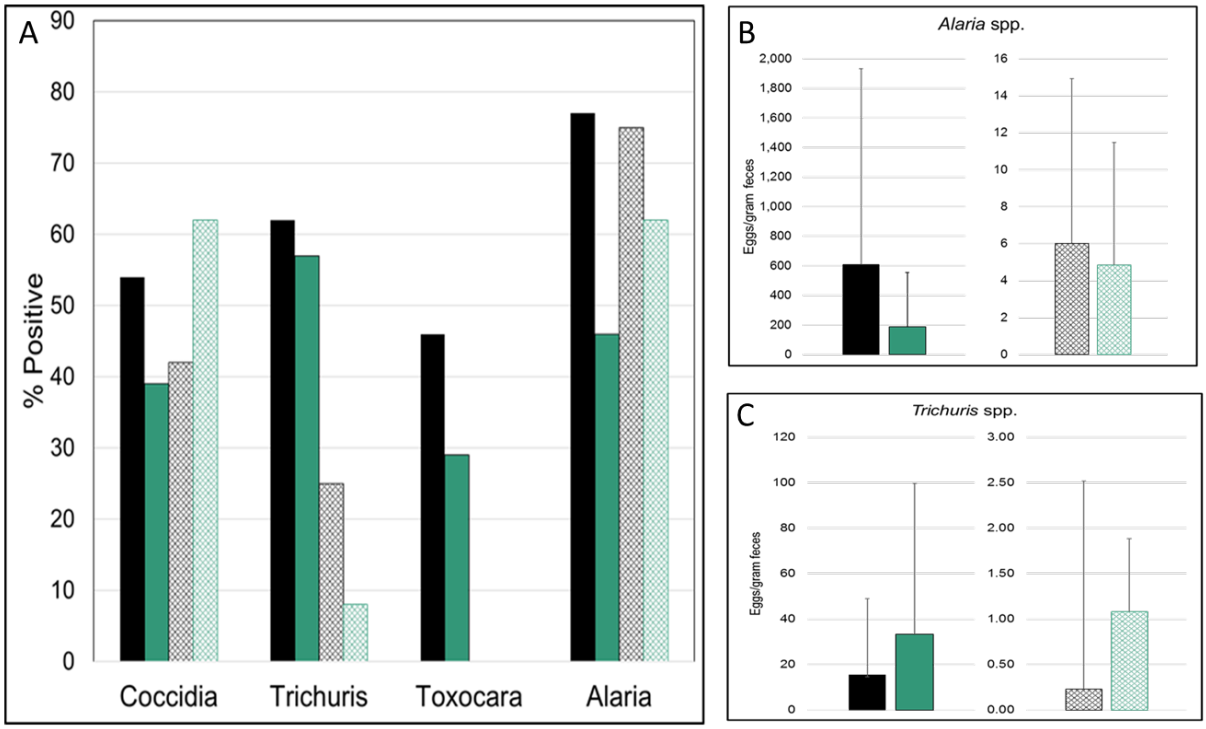

Figure 3.1. (A) Percent fecal samples testing positive for four common gastrointestinal parasite groups in wolf (solid bars) and raccoon dog (shaded bars) samples. Black bars indicate samples that were collected in regions with high (>3000 Bq/kg) levels of radiation contamination. Green bars indicate samples collected in regions with low (<800 Bq/kg) levels of radiation contamination. Number of eggs per gram feces for (B) *Alaria* spp and (C) *Trichuris* spp. Error bars represent 1 standard deviation.

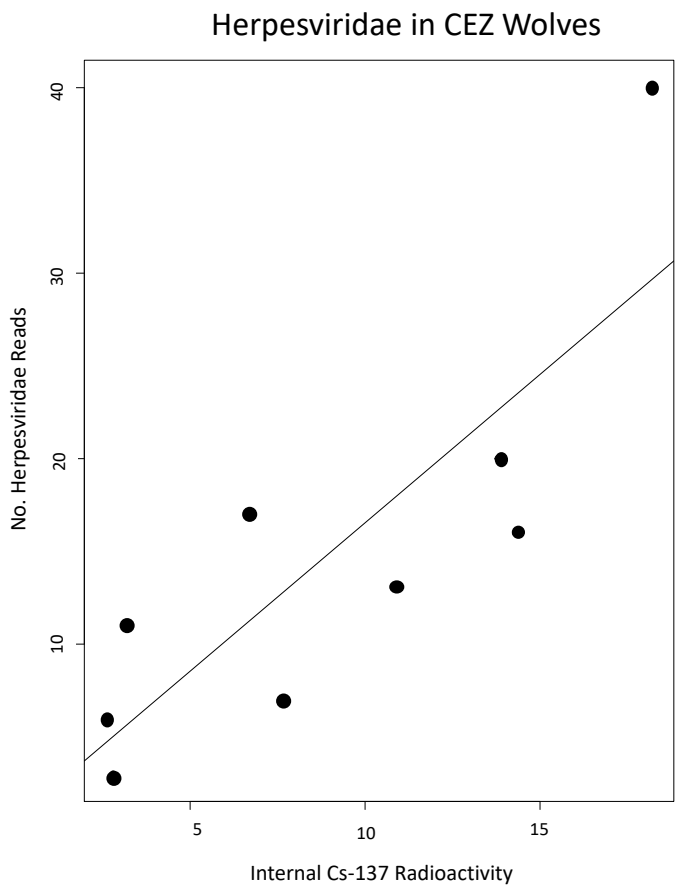

Figure 3.2. Internal Cs-137 activity rates and Herpesviruse transcription levels in wolves from the CEZ.

CHAPTER 4

SIGNATURES OF ENDOGENOUS RETROVIRAL-ENVIRONMENT INTERACTIONS IN WOLVES EXPOSED TO ENVIRONMENTAL IONIZING RADIATION FROM THE CHERNOBYL EXCLUSION ZONE[3]

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ABSTRACT

Endogenous retroviruses (ERVs) play an important role in vertebrate health and are associated with numerous neurological, autoimmune, and oncogenic diseases in humans, however the mechanisms driving ERV activation are largely unknown. ERVs can be regulated through epigenetic mechanisms, many of which can be altered by environmental stimuli. Here we examined global ERV transcription in wolves experiencing a gradient of ionizing radiation exposures in and around the Chernobyl Exclusion Zone. We found more ERV sites were activated in wolves from the CEZ as compared to reference individuals, and that expression levels of >90% of the up regulated ERVs positively correlated with internal radiocesium activity. Additionally, we found global ERV expression to be positively correlated with internal radiocesium activity and with expression of genes within three immune pathways (Cytokine receptor pathway, B-cell receptor pathway, and Fc gamma R-mediated phagocytosis pathway), and a pathway associated with transcriptional misregulation in cancer. Lastly, we identified the genes most proximal to the ten up regulated ERVs most strongly correlated with internal radiocesium activity. Eight of these proximal genes were up regulated in response to ionizing radiation and are associated with oncogenesis in humans. Our data suggest a significant effect of environmental ionizing radiation exposure on ERV activation and highlight the need for further research to fully describe the physiological implications of these interactions.

INTRODUCTION

Retroviral-host interactions influence host genomic structure and function and have significant impacts on host plasticity, health, and fitness (Eiden, 2008; Grandi & Tramontano, 2018). When retroviral sequences are inserted into the germline, they can be vertically

transmitted and become endogenous retroviruses (ERVs). Subsequently, ERV expression may be regulated by epigenetic control mechanisms such as DNA methylation or histone modifications (Li & Karlsson, 2016). ERV expression is often associated with tumor development, autoimmune disease, or neurological diseases (Gonzalez-Cao et al., 2016), but in some cases ERV proteins induce immune responses potentially beneficial in combating oncogenic cells (Bannert et al., 2018; Zhao et al., 2017).

Although ERV expression is associated with some diseases, not all ERV expression is detrimental. Highly active ERV promotors can act as bi-directional promoters of gene activation (Domansky et al., 2000) and thus contribute to gene regulation in the host genome. For example, ERVs are integral in placental morphogenesis for several species including humans (Blond et al., 2000; Mi et al., 2000), sheep (Dunlap et al., 2006), and mice (Gong et al., 2007). Additionally, other ERV elements are associated with beneficial immune response stimulation and in some cases this stimulation is dependent on ERVs. For example, in humans, stimulation of innate immune transcription networks in response to interferon stimulation is mediated by ERVs (Chuong, Elde, & Feschotte, 2016). Moreover, while many ERVs may no longer be fully functional viruses, they are still analogous to their exogenous counterparts. New research suggests expressed ERV elements are recognized in the cytoplasm and stimulate a retroviral immune responses which helps spur tumor cell apoptosis (Zhao et al., 2017; Bannert et al., 2018).

While ERVs can have multiple impacts on health, the mechanisms triggering activation are still not well understood. Many factors may influence ERV activation and are facilitated by binding of transcription factors to ERV long terminal repeat (LTR) promoters and enhancers outside of LTRs, genetic variation, and altered DNA methylation and/or histone modification

(reviewed in Li & Karlsson, 2016). During early embryogenesis most ERVs are rendered inactive by histone modifications and *de novo* DNA methylation (Rowe & Trono, 2011). DNA methylation is particularly key in restricting transcription of ERVs, with demethylation patterns leading to uncontrolled expression of ERV elements (Chen et al., 2003; Gaudet et al., 2004). Environmental factors, such as viral infection, can reactivate ERV elements (Perron & Lang, 2009). In particular, certain viruses can transactivate ERV promoters, as seen with the type W ERV family in humans (HERV-W) (Christensen et al., 2007; Koturbash, Pogribny, & Kovalchuk, 2005; Lafon et al., 2002). Environmentally induced HERV-W activation in multiple sclerosis patients is often associated with Herpesviridae infection (e.g. Epstein–Barr Virus, EBV; Human Herpes Virus type 6, HHV6; Herpes Simplex Virus type 1, HSV-1; and Varicella–Zoster Virus, VZV (Christensen et al., 2007; Ruprecht et al., 2006; Sotelo & Corona, 2011). Specifically, after interacting with the immune system in the brain, Herpesviridae viruses express specific genes that transactivate HERV-W and result in the production of Multiple Sclerosis-associated RetroViral virions (Perron et al., 1993; Perron & Lang, 2009). Additionally, influenza infection can transactivate HERV-W element promoters sensitive to viral and inflammatory triggers and increase the risk of developing schizophrenia (Perron et al., 2008; Stefansson et al., 2008). The increased expression of ERVs as a result of these infections may exert additional physiological effects that contribute to diseases associated with cellular misregulation and viral-associated tumors (Chen, Foroozesh, & Qin, 2019; Dai et al., 2018).

Recent research suggests that environmental stimuli may also play an important role in ERV activation (Balestrieri et al., 2018; Bernal et al., 2013; Cho, Lee, & Greenhalgh, 2008; Díaz-Carballo et al., 2017). For example, in humans, exposure to UV-radiation alters metabolic rates within melanoma cells, which leads to DNA hypomethylation and ERV expression

(Balestrieri et al., 2018). Similarly, the acute cellular stress observed at burn and septic sites exhibit increased ERV activation, potentially driven by the regulation of flanking host genes and gene networks associated with pathogenic responses to stress (Cho et al., 2008). Additionally, cytotoxic stress is linked to ERV transmission and transportation in cancer cells (Díaz-Carballo et al., 2017).

Exposure to ionizing radiation is an ideal model for examining ERV expression and potential environment-induced ERV-related disease. All species are exposed to natural and/or anthropogenic sources of ionizing radiation, which can alter immune function (Liu, 2003), induce carcinogenesis (Mavragani et al., 2017), alter epigenetic regulation of gene expression (Kovalchuk et al., 2004; Schofield & Kondratowicz, 2018), and impact ERV expression (Michna et al., 2016; Schanab et al., 2011). Ionizing radiation is frequently associated with hypomethylation and altered gene expression (Pogribny et al., 2004) that may be related to ERVs. For example, low ionizing radiation doses (1 and 3 cGy) can induce hypermethylation at a yellow Agouti (Avy) locus resulting in inactivation of an ERV that drives expression of the gene (Bernal et al., 2013). Interestingly, this pattern was mitigated in young Agouti by feeding antioxidants to the mother (Bernal et al., 2013), further suggesting interactive effects of environmental radiation exposure, subsequent induced stress, and antioxidant availability. All current studies on the impacts of ionizing radiation and ERVs come from laboratory or human radiation therapy studies leaving it unclear whether exposure under natural conditions can impact ERV activation and subsequent health effects.

Here we investigate the relationship between environmental exposure to ionizing radiation and ERV expression in a free ranging mammal. Our study examines gray wolves (*Canis lupus*) from the Chernobyl Exclusion Zone (CEZ) where we identify and quantify the

expression of ERVs in wolves with varying exposures to ionizing radiation and examine the potential relationship between ERV expression and immune function.

METHODS

Sample Collection

To collect samples from wolves experiencing a heterogeneous gradient of radiocesium (Cs-137) contamination, we live trapped wolves in the Belorussian CEZ using foothold traps in the fall and winter of 2014. Animal capture and handling was carried out in accordance with University of Georgia Animal Care and Use protocol A2015 05-004-Y2-A1. We collected blood samples from each individual and preserved them in RNAlater (Thermo Fisher Scientific) and stored them at -20°C until we transferred them to the University of Georgia's Savannah River Ecology Lab, USA where they were stored at -80°C. In the winter of 2014, we collaborated with hunting organizations to collect samples from wolves living in habitat similar to the CEZ, but with background radiation levels (hereafter BLR). Fresh blood samples were collected from hunted wolves and stored in the same manner as with trapped wolves. In addition, muscle and liver tissue samples were collected and stored at -20°C for Cs-137 quantification.

Radiocesium Quantification

We calculated Cs-137 exposure differently for live-trapped and hunted wolves. To estimate life-time radiation exposure for wolves trapped within the CEZ, we conducted Cs-137 body burden counts using a portable Cd-Zn-Te spectroscopy system as described in Hinton et al., (2019). For the BLR samples collected from hunted wolves, we used a Packard Cobra II auto-gamma counter (Model Cobra II 5003; Packard Instruments Co., Meriden, CT, USA) to quantify Cs-137 in desiccated and homogenized muscle tissues. For the counts, we used a NaI detector

and a ROI of 580e754 kiloelectron-Volts (keV) centered on 662 keV. Auto-calibration was conducted daily during the sample analysis using a traceable Cs-137 source (SREL-0113). We performed counts on each sample for 60 minutes and calculated background corrected Cs-137 count rates. We determined counter yield from matrix-specific standards with known Cs-137 quantities as described in Kennamer et al. (2017). We then calculated the minimum detectable concentrations (MDCs, Bq/g, dry mass) for each sample according to Currie (1968). To convert dry activity concentrations (Bq/g, dry weight) to wet activity concentrations (Bq/g, wet mass) we then used used wet:dry mass ratios.

Molecular Methods

Broadly, methods were as described in Chapter 2. But in brief, we isolated and purified total RNA using the RiboPure Blood Kit (Life Technologies, 2011) following the manufacturers' protocols. We then created libraries using the KAPA Stranded mRNA-seq kit (#KK4821) for 75 bp paired-end sequencing and incorporated individual barcodes. The samples were sequenced across four lanes on an Illumina NextSeq PE75 High Output Flow Cells (Georgia Genomics and Bioinformatics Core, University of Georgia).

ERV Identification and Analysis

Raw sequences were trimmed using Trimmomatic (Bolger, Lohse, & Usadel, 2014) to remove adapters and low-quality bases (quality < 20) with a window size of 5 and a minimum final size of > 60% initial read length. After completing quality control, we used various complimentary approaches for assessing ERV expression patterns in grey wolves from the CEZ.

We identified ERVs in each individual wolf transcriptome by referencing transcripts to the gEVE database of endogenous viral element open reading frames (ERV) in the CanFam3.1 genome (Nakagawa & Takahashi, 2016). This database was created to detect genomic regions

derived from viral infections by combining screening results of the CanFam3.1 genome using multiple viral identification programs (RetroTector (Sperber et al., 2007), RepeatMasker (Smit, Hubley, & Green, 2015) with RMblast and RepBase). ERVs were positively identified if it possessed an open reading frame longer than 80 amino acids and matched functional viral motif sequences archived in Pfam and Gypsy databases (Nakagawa & Takahashi, 2016). While utilizing this database, we created a custom reference database for NCBI's Magicblast (v1.4.0, Boratyn et al., 2019) and assessed transcriptomic sequences mapped from each wolf. To reduce the likelihood of false positives, we considered ERV regions to be expressed if they had > 89% mapping success and consisted of ≥ 2.0 counts per million in ≥ 6 samples.

After identifying ERVs in individuals we used several approaches to assess the potential relationship between radiation exposure and ERVs. Initially, we used an ANOVA to assess population level differences in the total number of expressed ERV regions between CEZ and BLR individuals, and then compared overall expression using a Mann-Whitney test. Then, at the individual level, we performed false discovery rate corrected spearman correlations between each ERV transcript count and individual wolf Cs-137 body burdens using *psych* package in R (Revelle, 2019). These correlations were performed on counts per million of each ERV to account for inter-library variation.

To examine differential expression of ERVs between wolves from the CEZ and BLR we utilized edgeR (Robinson, McCarthy, & Smyth, 2009). While running these analyses we controlled for library size using edgeR's *calcNormFactors* function and all further analyses were performed on these normalized reads. We used spearman correlations, as described above, to examine the relationship between expression of these up-regulated ERVs and wolf Cs-137 body burdens. To then visualize the location of the ERVs expressed in our individuals we constructed

a Manhattan plot of all ERVs using the *qqman* function in R (Turner 2014) while highlighting significantly up-regulated ERVs that were positively correlated with Cs-137 body burdens utilizing an adjusted significance threshold of $p < 0.0005$.

To assess potential relationships between ERV expression and immunological responses we calculated an eigengene expression equivalent (Alter, Brown, & Botstein, 2000) of first principal component (PC1) for ERV regulatory variation within each individual. We then used correlations to examine the relationship between these ERV eigengene equivalents (ERVEEs) and expression of immune function genes from four pathways: cytokine receptor, Fc gamma R-mediated phagocytosis, B cell receptor signaling, and natural killer cell receptor (Chapter 2, Table 4.2). Lastly, to assess more detailed correlations between the top ten ERVs correlated with radiation body burden and immune gene expression patterns, we again performed false discover rate corrected pairwise spearman correlations with each of these ERVEEs and the genes in the immune pathways assessed above. (Table 4.2).

RESULTS

We were able to examine ERV expression profiles in eighteen wolves: nine each from the CEZ and BLR. As expected, exposure history to Cs-137 differed in wolves from CEZ (8.89 ± 5.71 SD; range 3.1 – 18.2 kBq kg^{-1}) and BLR (0.06 ± 0.04 SD; range 0.03 – 0.11 kBq kg^{-1}) (Table 4.1). Overall, the number of total expressed ERV regions from magicblast alignments prior to and post low read count filtering were 11,121 and 10,996 respectively. Location of collection explained a significant amount of the variation in total number of expressed ERVs and post-hoc Mann-Whitney comparison shows that CEZ wolves had more expressed ERV sites ($p = 0005$). As can be seen in the principal component analysis, overall expression patterns of wolves

clearly cluster by location of origin, particularly along the PC1 axis which is associated with internal radiocesium activity (Figure 1).

Not only did the number of expressed ERVs differ among locations, but there was also evidence of divergent ERV regulation between radiation exposed and unexposed individuals. Overall there were 3,230 ERVs significantly differentially expressed in wolves from the two locations. Of these, 1,830 and 1,887 regions were significantly up-regulated and down-regulated (Figure 2) in wolves from the CEZ. Importantly, not only was expression different across locations, but even within the CEZ, expression of 96.9% (1,774 ERV) of the up-regulated regions was correlated with radiation exposure (evaluated with a FDR significance level of $p \leq 0.05$). These differentially expressed ERVs did not appear to show a chromosomal bias and were distributed throughout the genome (Figure 3).

To help elucidate if ERV expression may be related to individual physiology or health, we compared ERV expression patterns with expression of recognized innate and acquired immune pathways. ERV expression profiles were associated with body burden ($p = 0.005$) and transcription and immune pathways (cytokine receptor $p < 0.001$, Fc gamma R-mediated phagocytosis $p = 0.003$, B cell receptor signaling $p = 0.001$, and transcriptional misregulation in cancer $p = 0.005$). The ten up-regulated ERVs most strongly correlated with radiation body burden were also significantly correlated with >60% of genes from the four immune pathways examined (Table 4.2). Each of these ERVs fell in close proximal distance to ten genes with known cancer associations, and eight (SLC16A7, AKAP7, PCDH17, EFNB2, THAP9, CASP12, TERF2IP, KLHL13) of these are also upregulated in wolves from the CEZ (Chapter 2, Figure 5).

DISCUSSION

Increased ERV transcription is associated with cancer, neurological, and autoimmune diseases (Christensen et al., 2007; Gonzalez-Cao et al., 2016; Perron et al., 1993; Slokar & Hasler, 2016). While all ERVs may not be causative disease agents, many expressed ERVs are closely correlated with detrimental health impacts, particularly cancers. In this study we provide the first exploration of ERV transcription in free ranging individuals from a landscape contaminated with ionizing radiation. Further, we show that ERV transcription levels may serve as a bioindicator of long-term health impacts of chronic exposure to ionizing radiation.

Here, we not only see a clear difference in total ERV elements activated between wolves with and without exposure to radiation, but also see expression is strongly correlated with internal ionizing radioactivity within wolves from the CEZ. This suggests there are divergent ERV regulatory patterns between radiation exposed and unexposed individuals, as well as a regulatory dose response. We recognize that most of our results are correlational, but they do indicate a likely ERV-by-environment interaction with potentially significant impacts on individual health. Our study did not directly address the mechanisms that may have led to increased ERV expression. However, previous studies have demonstrated that environmental exposure to anthropogenic contaminants is associated with global patterns of hypomethylation (Nilsen et al., 2016). Methylation patterns are known to influence ERV expression (e.g. DNA methylation) and can be affected by ionizing radiation exposure (Kovalchuk et al., 2004). Thus, it is possible that altered methylation is driving the regulatory patterns we observed and is worth further study.

In humans, transactivation of ERVs can produce functional products and influence carcinogenesis by expression of viral mRNA, functional proteins, viral particles, and/or

circuitously activating oncogenic genes (Chen et al., 2019). Given this potential for transactivation of ERV elements and adjacent genes, we examined the types of ERVs most closely correlated with radiation exposure, as well as proximal genes of ERVs of interest. The ERVs we found to be expressed most closely resembled multiple retroviral sequences (Table 4.2), with no one viral type clearly dominating which ERVs correlated with radiation body burden.

Interestingly, the top ten upregulated ERVs correlated with radiation exposure were in close proximity to multiple genes associated with cancers (Cottone et al., 2018; Heery et al., 2017; Li et al., 2020; Liu et al., 2019; Pertega-Gomes et al., 2015). Expression of two of these genes, TBXT and PCDH17, was not detected in our data. The other eight proximal genes were up regulated in our wolves and are related to oncogenesis. All genes are described in Table 4.2, but in brief, aberrant upregulation of SLC16A7 (Pertega-Gomes et al., 2015), EFNB2 (Ni et al., 2020), THAP9 (Li et al., 2020; Majumdar, Singh, & Rio, 2013) and KLHL13 (Reddy, Khora, & Id, 2019) genes are associated with various cancers, PBX1 and AKAP7 genes induce fibroblast transformations at times associated with leukemia (Kamps, 1997; Mello et al., 2011; Thiaville et al., 2012), and the last two genes may confer beneficial characteristics. In particular, CASP12 expression is associated with increased patient survival from cervical cancer (Feng et al., 2019) and TERF2IP is a telomere regulating gene which plays a critical role in telomere protection, chromosomal stability and regulation of telomere length (Robles-espinoza et al., 2015; Slavutsky, 2017) and prevents telomere recombination and fragility. Ionizing radiation exposure is widely recognized to cause cancer(Moysich, Menezes, & Michalek, 2002). The ERV expression patterns we observed in wolves with elevated levels of internal ionizing radioactivity, as well as the patterns of the potentially transactivated genes in proximal distance to the top ten

up regulated ERVs, suggest there are further ERV by environment regulatory interactions and impacts on health which should be investigated.

Interestingly, the genes in the immune pathways examined also correlated with radiation body burden. It is entirely possible that exposure to ionizing radiation has independent effects on both ERV and immune pathway expression. However, it raises the question of whether ERV activation is stimulating an immune response which helps mitigate increased cancer rates associated with radiation exposure. Recent research has suggested ERV expression might promote tumor escape through immune modulation (Kudo-Saito et al., 2014; Mangeney et al., 2005). Additionally, some ERV activation may resemble exogenous retroviral infection closely enough to elicit an early immune response which drives the tumor cell into apoptosis (Bannert et al., 2018).

Our findings suggest there are significant interactive effects between ERVs and environmental radiation exposure which may influence oncogenic impacts of living in a radiation contaminated habitat. This study only amplifies the need for further research on the long-term health implications of environmental radiation contamination and mechanisms driving oncogenesis and cellular cancer mitigation in these environments.

REFERENCES

Alter, O., Brown, P. O., & Botstein, D. (2000). Singular value decomposition for genome-Wide expression data processing and modeling. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(18), 10101–10106. https://doi.org/10.1073/pnas.97.18.10101

Balestrieri, E., Argaw-Denboba, A., Gambacurta, A., Cipriani, C., Bei, R., Serafino, A., … Matteucci, C. (2018). Human endogenous retrovirus K in the crosstalk between cancer cells microenvironment and plasticity: A new perspective for combination therapy. *Frontiers in Microbiology*, *9*(JUL), 1–8. https://doi.org/10.3389/fmicb.2018.01448

Bannert, N., Hofmann, H., Block, A., & Hohn, O. (2018). HERVs new role in cancer: From accused perpetrators to cheerful protectors. *Frontiers in Microbiology*, *9*(FEB), 1–8. https://doi.org/10.3389/fmicb.2018.00178

Bernal, A. J., Dolinoy, D. C., Huang, D., Skaar, D. A., Weinhouse, C., & Jirtle, R. L. (2013). Adaptive radiation-induced epigenetic alterations mitigated by antioxidants. *FASEB Journal*, *27*(2), 665–671. https://doi.org/10.1096/fj.12-220350

Blond, J.-L., Lavillette, D., Cheynet, V., Bouton, O., Oriol, G., Chapel-Fernandes, S., … Cosset, F.-L. (2000). An Envelope Glycoprotein of the Human Endogenous Retrovirus HERV-W Is Expressed in the Human Placenta and Fuses Cells Expressing the Type D Mammalian Retrovirus Receptor. *Journal of Virology*, *74*(7), 3321–3329. https://doi.org/10.1128/jvi.74.7.3321-3329.2000

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Boratyn, G. M., Thierry-Mieg, J., Thierry-Mieg, D., Busby, B., & Madden, T. L. (2019). Magic-BLAST, an accurate RNA-seq aligner for long and short reads. *BMC Bioinformatics*, *20*(1), 1–19. https://doi.org/10.1186/s12859-019-2996-x

Chen, J., Foroozesh, M., & Qin, Z. (2019). Transactivation of human endogenous retroviruses by tumor viruses and their functions in virus-associated malignancies. *Oncogenesis*, *8*(1). https://doi.org/10.1038/s41389-018-0114-y

Chen, T., Ueda, Y., Dodge, J. E., Wang, Z., & Li, E. (2003). Establishment and Maintenance of Genomic Methylation Patterns in Mouse Embryonic Stem Cells by Dnmt3a and Dnmt3b. *Molecular and Cellular Biology*, *23*(16), 5594–5605. https://doi.org/10.1128/mcb.23.16.5594-5605.2003

Cho, K., Lee, Y. K., & Greenhalgh, D. G. (2008). Endogenous retroviruses in systemic response to stress signals. *Shock*, *30*(2), 105–116. https://doi.org/10.1097/SHK.0b013e31816a363f

Christensen, T., Petersen, T., Thiel, S., Brudek, T., Ellermann-Eriksen, S., & Møller-Larsen, A. (2007). Gene-environment interactions in multiple sclerosis: Innate and adaptive immune responses to human endogenous retrovirus and herpesvirus antigens and the lectin complement activation pathway. *Journal of Neuroimmunology*, *183*(1–2), 175–188. https://doi.org/10.1016/j.jneuroim.2006.09.014

Chuong, E. B., Elde, N. C., & Feschotte, C. (2016). Regulatory evolution of innate immunity through co-option of endogenous retroviruses. *Science*, *351*(6277), 1083–1087. https://doi.org/10.1126/science.aad5497

Cottone, L., Hookway, E. S., Cribbs, A., Wells, G., Lombard, P., Ligammari, L., … Oppermann, U. (2018). Epigenetic inactivation of oncogenic brachyury (TBXT) by H3K27 histone demethylase controls chordoma cell survival. *BioRxiv*, *44*(0), 1–22.

Currie, L. A. (1968). Limits for Qualitative Detection and Quantitative Determination: Application to Radiochemistry. *Analytical Chemistry*, *40*(3), 586–593. https://doi.org/10.1021/ac60259a007

Dai, L., Del Valle, L., Miley, W., Whitby, D., Ochoa, A. C., Flemington, E. K., & Qin, Z. (2018). Transactivation of human endogenous retrovirus K (HERV-K) by KSHV promotes Kaposi's sarcoma development. *Oncogene*, *37*(33), 4534–4545. https://doi.org/10.1038/s41388-018-0282-4

Díaz-Carballo, D., Klein, J., Acikelli, A. H., Wilk, C., Saka, S., Jastrow, H., … Strumberg, D. (2017). Cytotoxic stress induces transfer of mitochondria-associated human endogenous retroviral rna and proteins between cancer cells. *Oncotarget*, *8*(56), 95945–95964. https://doi.org/10.18632/oncotarget.21606

Domansky, A. N., Kopantzev, E. P., Snezhkov, E. V., Lebedev, Y. B., Leib-Mosch, C., & Sverdlov, E. D. (2000). Solitary HERV-K LTRs possess bi-directional promoter activity and contain a negative regulatory element in the U5 region. *FEBS Letters*, *472*(2–3), 191–195. https://doi.org/10.1016/S0014-5793(00)01460-5

Dunlap, K. A., Palmarini, M., Varela, M., Burghardt, R. C., Hayashi, K., Farmer, J. L., & Spencer, T. E. (2006). Endogenous retroviruses regulate periimplantation placental growth and differentiation. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(39), 14390–14395. https://doi.org/10.1073/pnas.0603836103

Eiden, M. V. (2008). Endogenous retroviruses: Endogenous retroviruses - Aiding and abetting genomic plasticity. *Cellular and Molecular Life Sciences*, *65*(21), 3327–3328. https://doi.org/10.1007/s00018-008-8493-4

Feng, G., Beilei, Z., Caizhi, C., & Wen, Z. (2019). Analysis of CASP12 diagnostic and

prognostic values in cervical cancer based on TCGA database. *Bioscience Reports*, *39*(December), 1–11.

Gaudet, F., Rideout, W. M., Meissner, A., Dausman, J., Leonhardt, H., & Jaenisch, R. (2004). Dnmt1 Expression in Pre- and Postimplantation Embryogenesis and the Maintenance of IAP Silencing. *Molecular and Cellular Biology*, *24*(4), 1640–1648. https://doi.org/10.1128/mcb.24.4.1640-1648.2004

Gong, R., Huang, L., Shi, J., Luo, K., Qiu, G., & Feng, H. (2007). Cellular Physiology Biochemistry and Biochemistr y Syncytin-A Mediates the Formation of Syncytiotrophoblast Involved in Mouse Placental Development. *Cellular Physiology and Biochemistry*, *20*, 517–526.

Gonzalez-Cao, M., Iduma, P., Karachaliou, N., Santarpia, M., Blanco, J., & Rosell, R. (2016). Human endogenous retroviruses and cancer. *Cancer Biology and Medicine*, *13*(4), 483–488. https://doi.org/10.20892/j.issn.2095-3941.2016.0080

Grandi, N., & Tramontano, E. (2018). Human endogenous retroviruses are ancient acquired elements still shaping innate immune responses. *Frontiers in Immunology*, *9*(SEP), 1–16. https://doi.org/10.3389/fimmu.2018.02039

Heery, C. R., Palena, C., McMahon, S., Donahue, R. N., Lepone, L. M., Grenga, I., … Gulley, J. L. (2017). Phase I study of a poxviral TRICOM-based vaccine directed against the transcription factor brachyury. *Clinical Cancer Research*, *23*(22), 6833–6845. https://doi.org/10.1158/1078-0432.CCR-17-1087

Hinton, T. G., Byrne, M. E., Webster, S. C., Love, C. N., Broggio, D., Trompier, F., … Beasley, J. C. (2019). GPS-coupled contaminant monitors on free-ranging Chernobyl wolves challenge a fundamental assumption in exposure assessments. *Environment International*,

133(July), 105152. https://doi.org/10.1016/j.envint.2019.105152

Kamps, M. P. (1997). *E2a-Pbx1 Induces Aberrant Expression of Tissue-Specific and Developmentally Regulated Genes When Expressed in NIH 3T3 Fibroblasts*. *17*(3), 1503–1512.

Kennamer, R. A., Oldenkamp, R. E., Leaphart, J. C., King, J. D., Bryan, A. L., & Beasley, J. C. (2017). Radiocesium in migratory aquatic game birds using contaminated U.S. Department of Energy reactor-cooling reservoirs: A long-term perspective. *Journal of Environmental Radioactivity*, *171*, 189–199. https://doi.org/10.1016/j.jenvrad.2017.02.022

Koturbash, I., Pogribny, I., & Kovalchuk, O. (2005). Stable loss of global DNA methylation in the radiation-target tissue - A possible mechanism contributing to radiation carcinogenesis? *Biochemical and Biophysical Research Communications*, *337*(2), 526–533. https://doi.org/10.1016/j.bbrc.2005.09.084

Kovalchuk, I., Abramov, V., Pogribny, I., Kovalchuk, O., Physiology, S. P., & May, N. (2004). Molecular Aspects of Plant Adaptation to Life in the Chernobyl Zonel [w]. *Plant Physiology*, *135*(1), 357–363. https://doi.org/10.1104/pp.104.040477.stresses

Kudo-Saito, C., Yura, M., Yamamoto, R., & Kawakami, Y. (2014). Induction of immunoregulatory CD271+ cells by metastatic tumor cells that express human endogenous retrovirus H. *Cancer Research*, *74*(5), 1361–1370. https://doi.org/10.1158/0008-5472.CAN-13-1349

Lafon, M., Jouvin-Marche, E., Marche, P. N., Perron, H., & Woodland, D. L. (2002). Human viral superantigens: To be or not to be transactivated? *Trends in Immunology*, *23*(5), 238–239. https://doi.org/10.1016/S1471-4906(02)02207-X

Li, F., & Karlsson, H. (2016). Expression and regulation of human endogenous retrovirus W

elements. *Apmis*, *124*(1–2), 52–66. https://doi.org/10.1111/apm.12478

Li, N., Yang, G., Luo, L., Ling, L., Wang, X., Shi, L., … Zheng, G. (2020). LncRNA THAP9-AS1 promotes pancreatic ductal adenocarcinoma growth and leads to a poor clinical outcome via sponging miR-484 and interacting with YAP. *Clinical Cancer Research*, *26*(7), 1736–1748. https://doi.org/10.1158/1078-0432.CCR-19-0674

Life Technologies. (2011). RiboPure™-Blood Kit Protocol (PN 1928M Rev D). *Protocol*.

Liu, S.-Z. (2003). Nonlinear dose-response relationship in the immune system following exposure to ionizing radiation: mechanisms and implications. *Nonlinearity in Biology, Toxicology, Medicine*, *1*(1), 71–92.

Liu, S., Lin, H., Wang, D., Li, Q., Luo, H., Li, G., … Liu, Y. (2019). PCDH17 increases the sensitivity of colorectal cancer to 5- fl uorouracil treatment by inducing apoptosis and autophagic cell death. *Signal Transduction and Targeted Therapy*, (September). https://doi.org/10.1038/s41392-019-0087-0

Majumdar, S., Singh, A., & Rio, D. C. (2013). The Human THAP9 Gene Encodes an Active. *Science*, *339*(January), 446–449.

Mangeney, M., Pothlichet, J., Renard, M., Ducos, B., & Heidmann, T. (2005). Endogenous retrovirus expression is required for murine melanoma tumor growth in vivo. *Cancer Research*, *65*(7), 2588–2591. https://doi.org/10.1158/0008-5472.CAN-04-4231

Mavragani, I. V., Nikitaki, Z., Souli, M. P., Aziz, A., Nowsheen, S., Aziz, K., … Georgakilas, A. G. (2017). Complex DNA damage: A route to radiation-induced genomic instability and carcinogenesis. *Cancers*, *9*(7), 1–22. https://doi.org/10.3390/cancers9070091

Mello, S. S., Fachin, A. L., Junta, C. M., Sandrin-garcia, P., Donadi, E. A., Passos, G. A. S., & Gene, D. De. (2011). *Delayed Effects of Exposure to a Moderate Radiation Dose on*

Transcription Profiles in Human Primary Fibroblasts. *129*. https://doi.org/10.1002/em

Mi, S., Lee, X., Li, X. ping, Veldman, G. M., Finnerty, H., Racie, L., … McCoy, J. M. (2000). Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature*, *403*(6771), 785–789. https://doi.org/10.1038/35001608

Michna, A., Schötz, U., Selmansberger, M., Zitzelsberger, H., Lauber, K., Unger, K., & Hess, J. (2016). Transcriptomic analyses of the radiation response in head and neck squamous cell carcinoma subclones with different radiation sensitivity: Time-course gene expression profiles and gene association networks. *Radiation Oncology*, *11*(1), 1–16. https://doi.org/10.1186/s13014-016-0672-0

Moysich, K. B., Menezes, R. J., & Michalek, A. M. (2002). Chernobyl-related ionising radiation exposure and cancer risk: An epidemiological review. *Lancet Oncology*, *3*(5), 269–279. https://doi.org/10.1016/S1470-2045(02)00727-1

Nakagawa, S., & Takahashi, M. U. (2016). gEVE: a genome-based endogenous viral element database provides comprehensive viral protein-coding sequences in mammalian genomes. *Database : The Journal of Biological Databases and Curation*, *2016*, 1–8. https://doi.org/10.1093/database/baw087

Ni, Q., Chen, P., Zhu, B., Li, J., Xie, D., & Ma, X. (2020). Expression levels of EPHB4 , EFNB2 and caspase - 8 are associated with clinicopathological features and progression of esophageal squamous cell cancer. *Oncology Letters*, *19*, 917–929. https://doi.org/10.3892/ol.2019.11160

Nilsen, F. M., Parrott, B. B., Bowden, J. A., Kassim, B. L., Somerville, S. E., Bryan, T. A., … Guillette, L. J. (2016). Global DNA methylation loss associated with mercury contamination and aging in the American alligator (Alligator mississippiensis). *Science of*

The Total Environment, *545–546*, 389–397. https://doi.org/10.1016/j.scitotenv.2015.12.059

Perron, H., Suh, M., Lalande, B., Gratacap, B., Laurent, A., Stoebner, P., & Seigneurin, J. R. (1993). Herpes simplex virus ICP0 and ICP4 immediate early proteins strongly enhance expression of a retrovirus harboured by a leptomeningeal cell line from a patient with multiple sclerosis. *Journal of General Virology*, *74*(1), 65–72. https://doi.org/10.1099/0022-1317-74-1-65

Perron, Hervé, & Lang, A. (2009). The human endogenous retrovirus link between genes and environment in multiple sclerosis and in multifactorial diseases associating neuroinflammation. *Clinical Reviews in Allergy and Immunology*, *39*(1), 51–61. https://doi.org/10.1007/s12016-009-8170-x

Perron, Hervé, Mekaoui, L., Bernard, C., Veas, F., Stefas, I., & Leboyer, M. (2008). Endogenous Retrovirus Type W GAG and Envelope Protein Antigenemia in Serum of Schizophrenic Patients. *Biological Psychiatry*, *64*(12), 1019–1023. https://doi.org/10.1016/j.biopsych.2008.06.028

Pertega-Gomes, N., Vizcaino, J. R., Felisbino, S., Warren, A. Y., Shaw, G., Kay, J., … Massie, C. E. (2015). Epigenetic and oncogenic regulation of SLC16A7 (MCT2) results in protein over-expression , impacting on signalling and cellular phenotypes in prostate cancer. *Oncotarget*, *6*(25).

Pogribny, I., Raiche, J., Slovack, M., & Kovalchuk, O. (2004). Dose-dependence, sex- and tissue-specificity, and persistence of radiation-induced genomic DNA methylation changes. *Biochemical and Biophysical Research Communications*, *320*(4), 1253–1261. https://doi.org/10.1016/j.bbrc.2004.06.081

Qubit. (2007). Qubit® Fluorometer: Instruction Manual. *Qubit® Fluorometric Quantification*.

Retrieved from http://www.invitrogen.com/etc/medialib/en/filelibrary/cell_tissue_analysis/Qubit-all-file-types.Par.27078.File.dat/Qubit_UserManual.pdf

Reddy, R. B., Khora, S. S., & Id, A. S. (2019). *Molecular prognosticators in clinically and pathologically distinct cohorts of head and neck squamous cell carcinoma — A meta-analysis approach*. 1–29.

Revelle, W. (2019). *psych:Procedures for Psychological, Psychometric, and Personality Research* (pp. 0–386). pp. 0–386. Retrieved from https://cran.r-project.org/package=psych

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2009). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, *26*(1), 139–140. https://doi.org/10.1093/bioinformatics/btp616

Robles-espinoza, C. D., Velasco-herrera, M. C., Hayward, N. K., & Adams, D. J. (2015). *Telomere-Regulating Genes and the Telomere Interactome in Familial Cancers*. 211–223. https://doi.org/10.1158/1541-7786.MCR-14-0305

Rowe, H. M., & Trono, D. (2011). Dynamic control of endogenous retroviruses during development. *Virology*, *411*(2), 273–287. https://doi.org/10.1016/j.virol.2010.12.007

Ruprecht, K., Obojes, K., Wengel, V., Gronen, F., Kim, K. S., Perron, H., … Rieckmann, P. (2006). Regulation of human endogenous retrovirus W protein expression by herpes simplex virus type 1: Implications for multiple sclerosis. *Journal of NeuroVirology*, *12*(1), 65–71. https://doi.org/10.1080/13550280600614973

Schanab, O., Humer, J., Gleiss, A., Mikula, M., Sturlan, S., Grunt, S., … Waltenberger, A. (2011). Expression of human endogenous retrovirus K is stimulated by ultraviolet radiation in melanoma. *Pigment Cell and Melanoma Research*, *24*(4), 656–665.

https://doi.org/10.1111/j.1755-148X.2011.00860.x

Schofield, P. N., & Kondratowicz, M. (2018). Evolving paradigms for the biological response to low dose ionizing radiation; the role of epigenetics. *International Journal of Radiation Biology*, *94*(8), 769–781. https://doi.org/10.1080/09553002.2017.1388548

Slavutsky, I. (2017). Shelterin genes, germ line mutations and chronic lymphocytic leukemia. *Translational Cancer Research*, *6*(11), 68–71. https://doi.org/10.21037/tcr.2017.02.36

Slokar, G., & Hasler, G. (2016). Human endogenous retroviruses as pathogenic factors in the development of schizophrenia. *Frontiers in Psychiatry*, *6*(JAN), 1–10. https://doi.org/10.3389/fpsyt.2015.00183

Smit, A., Hubley, R., & Green, P. (2015). *RepeatMasker Open-4.0*. Retrieved from http://www.repeatmasker.org

Sotelo, J., & Corona, T. (2011). Varicella Zoster Virus and Relapsing Remitting Multiple Sclerosis. *Multiple Sclerosis International*, *2011*, 1–5. https://doi.org/10.1155/2011/214763

Sperber, G. O., Airola, T., Jern, P., & Blomberg, J. (2007). Automated recognition of retroviral sequences in genomic data - RetroTector©. *Nucleic Acids Research*, *35*(15), 4964–4976. https://doi.org/10.1093/nar/gkm515

Stefansson, H., Rujescu, D., Cichon, S., Pietiläinen, O. P. H., Ingason, A., Steinberg, S., … Myin-Germeys, I. (2008). Large recurrent microdeletions associated with schizophrenia. *Nature*, *455*(7210), 232–236. https://doi.org/10.1038/nature07229

Thiaville, M. M., Stoeck, A., Chen, L., Wu, R., Magnani, L., Oidtman, J., … Wang, T. (2012). Identification of PBX1 Target Genes in Cancer Cells by Global Mapping of PBX1 Binding Sites. *PLoS ONE*, *7*(5), 1–10. https://doi.org/10.1371/journal.pone.0036054

Zhao, H., Ning, S., Nolley, R., Scicinski, J., Oronsky, B., Knox, S. J., & Peehl, D. M. (2017).

The immunomodulatory anticancer agent, RRx-001, induces an interferon response through epigenetic induction of viral mimicry. *Clinical Epigenetics*, *9*(1), 1–11. https://doi.org/10.1186/s13148-017-0312-z

TABLES

Table 4.1. Number of expressed ERV sites in wolves from the Chernobyl Exclusion Zone (CEZ) and northern Belarus (BLR) and the individual radiocesium (Cs-137) body burdens (kBq kg^{-1}).

Individual	Collection Location	Cs-137 Body Burden	No. Expressed ERVs
C1	CEZ	18.2	8894
C2	CEZ	13.8	6676
C3	CEZ	6.7	6463
C4	CEZ	14.3	7329
C5	CEZ	10.9	5081
C6	CEZ	3.1	5747
C7	CEZ	2.8	5606
C8	CEZ	2.6	7378
C9	CEZ	7.68	8868
N101	BLR	0.0303	7400
N102	BLR	0.0614	3486
N103	BLR	0.0382	3609
N104	BLR	0.1081	4260
N105	BLR	0.0467	3276
N106	BLR	0.0478	4006
N107	BLR	0.0372	5301
N108	BLR	0	4993
N109	BLR	0.1476	5848

Table 4.2. Descriptions of the top ten significantly up regulated ERV correlated with radiation body burden. This includes viral blast annotation for each ERV sequence (gEVE ref). Percent genes from four immune pathways (Cytokine receptor pathway, B-cell receptor pathway, Fc gamma R-mediated phagocytosis pathway, and natural killer cell mediated cytotoxicity pathway) which significantly associated with each ERV expression profile. And lastly, the closest adjacent gene to each ERV and descriptions of the gene family (as described by NCBI gene database accessed December 2019).

gEVE ERV ID	Viral Blast	Cytokine receptor pathway	B cell receptor signaling pathway	Fc gamma R-mediated phagocytosis pathway	Natural Killer Cell Receptor	Adjacent gene ID	Adjacent gene family description
Cfam31.chr1.54142232.54145222.+	Lymphocystis disease virus	71.79%	75.00%	65.15%	72.58%	Tbxt	Provides instructions for making T-box proteins
Cfam31.chr1.69585364.69585780.-	Woolly monkey sarcoma virus	73.08%	78.57%	69.70%	83.87%	AKAP7	A-kinase anchoring protein family proteins
Cfam31.chr10.3724071.3726731.+	Lymphocystis disease virus	66.67%	69.64%	45.45%	67.74%	SLC16A7	Monocarboxylate transporter family, transporting metabolites, such as lactate, pyruvate, and ketone bodies
Cfam31.chr22.13704155.13704502.+	Lymphocystis disease virus	66.67%	78.57%	68.18%	72.58%	Pcdh17	Protocadherin gene family, a subfamily of the cadherin superfamily
Cfam31.chr22.55037214.55037966.-	D-type betaretrovirida e	70.51%	80.36%	80.30%	79.03%	EFNB2	Member of the ephrin family, a subfamily of receptor protein-tyrosine kinases and have been implicated in mediating developmental events, especially in the nervous system and in erythropoiesis
Cfam31.chr32.707273.7037614.-	Lymphocystis disease virus	60.26%	76.79%	68.18%	74.19%	Thap9	part of a C2CH zinc-coordinating site-specific DNA binding domain
Cfam31.chr38.19016668.19017345.-	Lymphocystis disease virus	71.79%	91.07%	71.21%	82.26%	PBX1	PBX homeobox family of transcriptional factors

Cfam31.chr5.27315184.27315762.+	Koala retrovirus	61.54%	75.00%	65.15%	75.81%	CASP12	Most highly related to members of the ICE subfamily of caspases that process inflammatory cytokines
Cfam31.chr5.74993715.74994896.+	Moloney murine leukemia virus	93.59%	75.00%	69.70%	75.81%	TERF2IP	Part of a complex involved in telomere length regulation
Cfam31.chrX.89675046.89675552.+	Lymphocystis disease virus	88.46%	91.07%	71.21%	82.26%	KLHL13	Coordinates faithful mitotic progression and completion of cytokinesis

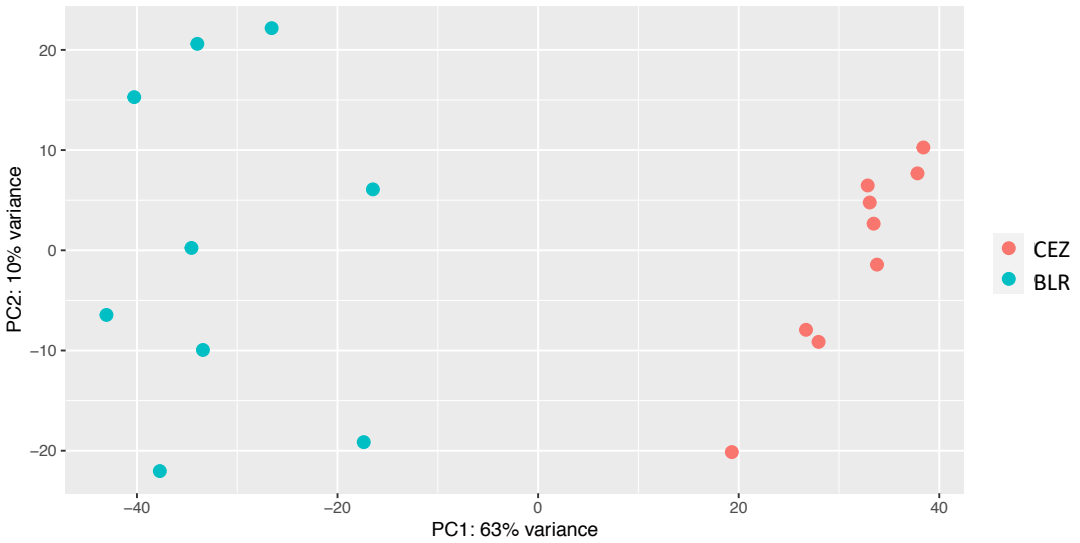

Figure 4.1. PCA plot of ERV expression patterns in wolves from the Chernobyl Exclusion Zone (CEZ) and northern Belarus (BLR).

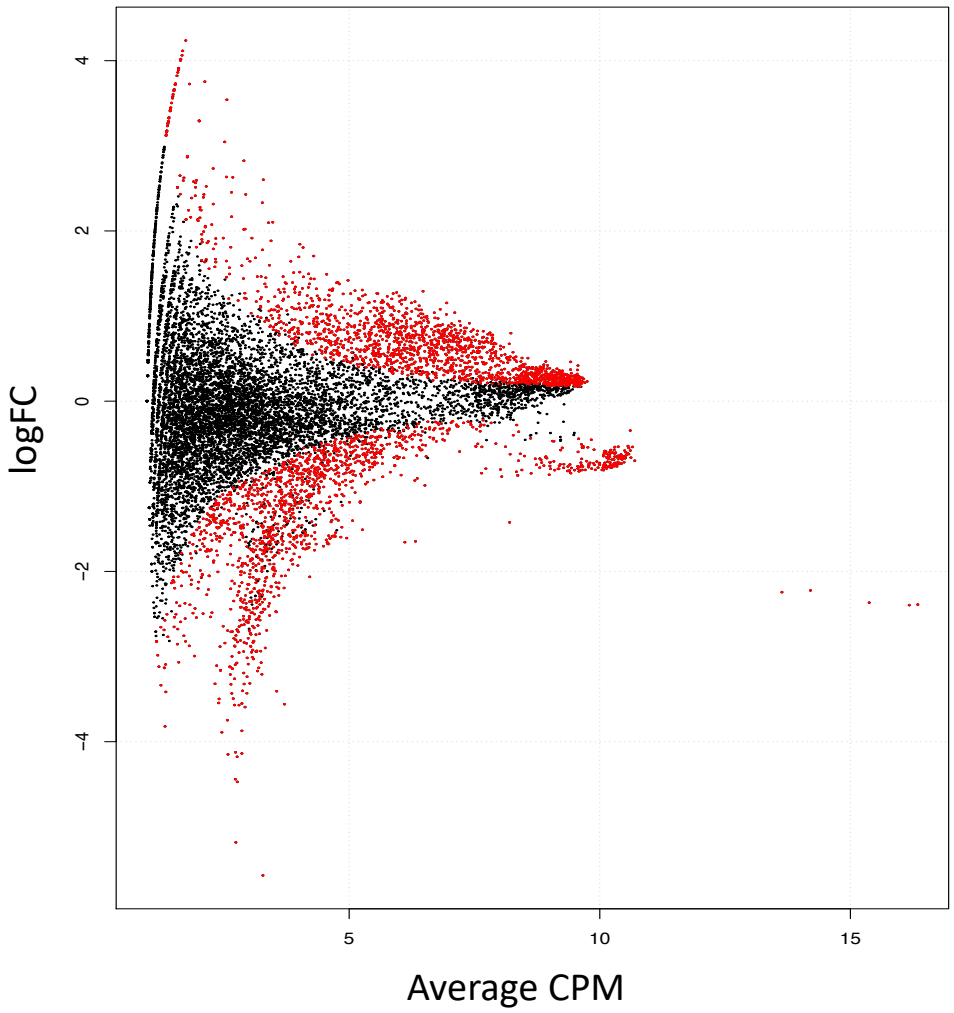

Figure 4.2. Smear plot of up and down regulated ERV regions in wolves from the CEZ. Significant differentially expressed genes are in red.

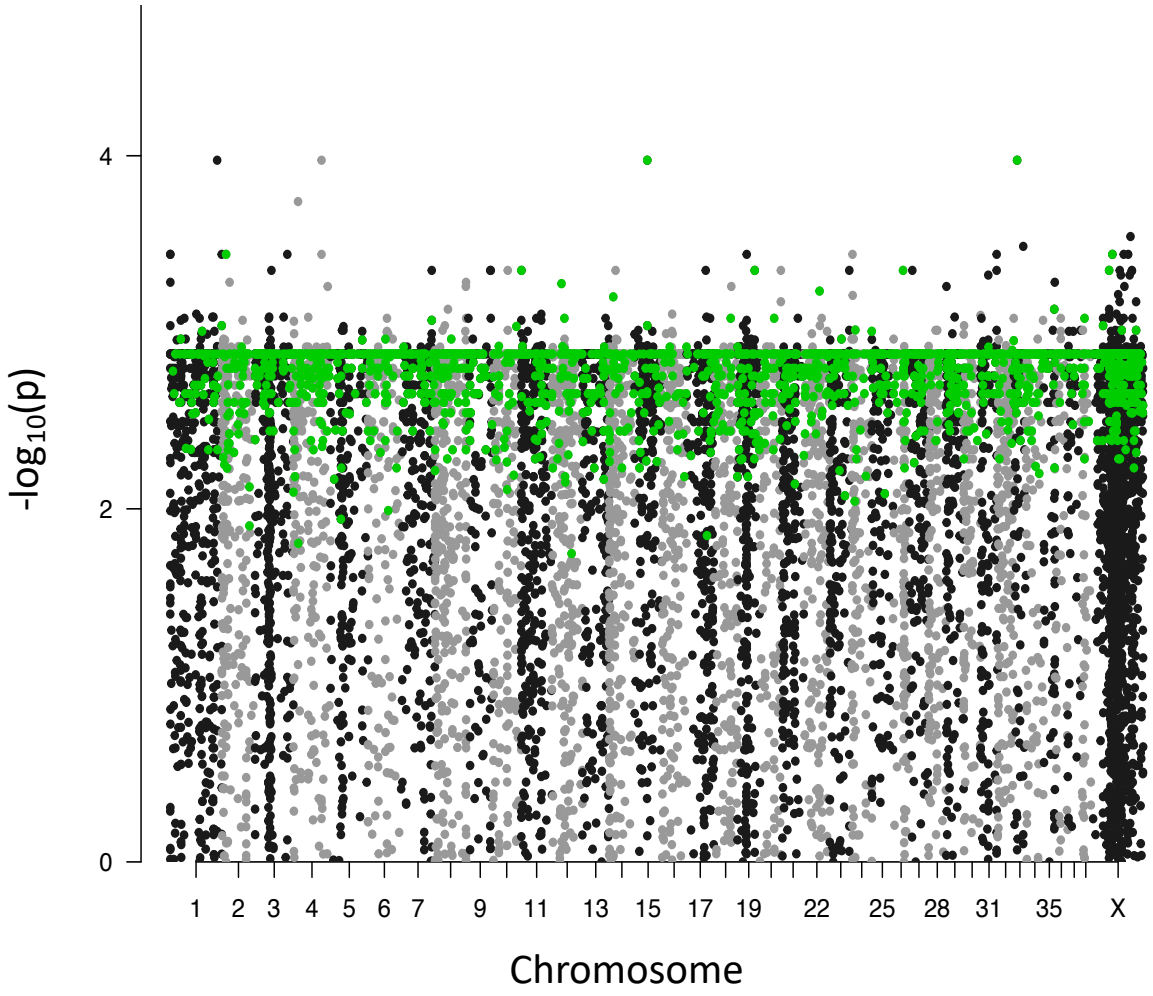

Figure 4.3. Manhattan plot of expressed ERVs in wolves from Belarus. Green points indicate significantly up regulated ERVs which also correlate with Cs-137 body burden.

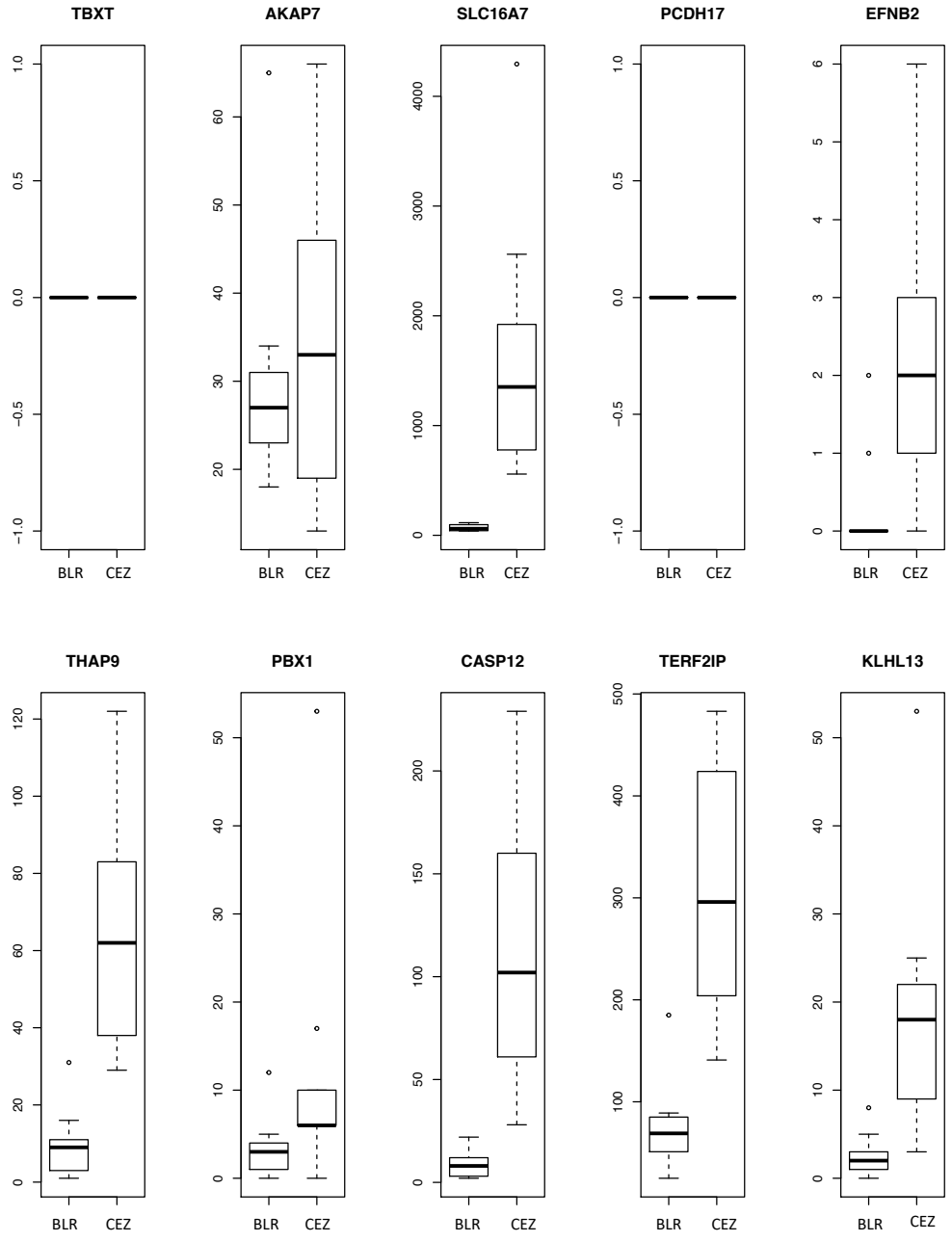

Figure 4.4: Expression profiles for genes that are in the closest proximal distance to the ten ERVs with the strongest positive correlation between expression level and radiation body burden. Raw gene transcript counts in wolves from northern Belarus (BLR) and the Chernobyl Exclusion Zone (CEZ).

CHAPTER 5

REGULATORY DIVERGENCE IN RACCOON DOGS EXPOSED TO ELEVATED LEVELS OF ENVIRONMENTAL IONIZING RADIATION HIGHLIGHT IMMUNOLOGICAL AND CELLULAR TRANSPORT EFFECTS OF EXPOSURE[4]

[4] Love, CN, SC Webster, D Shamovich, ME Byrne, PE Schlichting, JC Beasley, TG Hinton, and SL Lance. To be submitted to *TBD.*

ABSTRACT

Population persistence in the face of anthropogenically contaminated environments often results from acclimation at the individual level and/or adaptation at the population level. Both of these processes can be facilitated through altered regulation of gene pathways. Increasing demands on energy production is resulting in construction of additional nuclear power plants and an increased risk of environments contaminated with radiation. Here we performed *de novo* RNA sequencing assembly technique to examine whole transcriptome expression patterns in blood of raccoon dogs (*Nyctereutes procyonoides*) living in the ionizing radiation contaminated Chernobyl Exclusion Zone to examine mechanisms of acclimation to radiation exposure. We identified global expression patterns which diverged across the CEZ boundary. Of the 16,326 expressed genes, 1,099 were differentially expressed, with 644 up regulated and 455 down regulated in individuals living in the CEZ. We additionally implemented Weighted Gene Coexpression Network Analysis to identify co-expression modules within the blood transcriptome. Two expression modules were associated with site of origin and one module showed a site of origin by radiation affect. The most highly differentially expressed genes and top hub module genes are associated with immune response, cellular homeostasis, oncogenesis, and ion transport. These expression patterns not only describe detrimental impacts of radiation exposure but may also describe mechanisms facilitating mitigation of radiation induced cellular damage.

INTRODUCTION

In an era where human activities are driving ecosystem changes globally, wildlife are exposed to an increasing number of anthropogenic stressors (Otto, 2018; Subramanian, 2019) and environments that change at a rate not experienced in recent history. Subsequently, some populations have gone locally extinct, while others have persisted (Otto, 2018; Parmesan & Yohe, 2003; Stuart et al., 2004; Waterhouse et al., 2018). Population persistence often results from acclimation at the individual level (Donelson et al., 2012; Šrut, Drechsel, & Höckner, 2017) and/or adaptation at the population level (Hoffmann & Sgrò, 2011; Williams et al., 2008). Acclimation can be facilitated through altered regulation of gene pathways. For example, changes in transcriptional responses is associated with killifish tolerance to extreme osmotic conditions (Whitehead et al., 2011) and daphnia tolerance to cadmium (Shaw et al., 2019). Natural selection can act on individual gene expression variation, canalizing adaptive transcription patterns (Shaw et al., 2014), and leading to population adaptation. Examining transcriptional responses across various species provides critical insights into the regulatory networks underlying adaptation to exposure to anthropogenic stressors.

One of the leading ways humans impact ecosystems is through industrial contamination and there is abundant work examining acclimation and adaptation to chemical contaminants (Asselman et al., 2012; Flynn, Love, Coleman, & Lance, 2019; Reid et al., 2016). Many environmental contaminants are novel, while others are naturally occurring elements that become toxic when levels are elevated from agricultural and industrial practices. Ionizing radiation is a perfect example of this because most organisms experience natural exposure through things such as UV radiation and radon emissions. However, in radiation contaminated environments, due to uranium mining, nuclear energy production, or nuclear weapons testing, individuals experience

elevated levels (Lourenço, Mendo, & Pereira, 2016). At low levels ionizing radiation exposure is not toxic and many species have physiological mechanism in place for dealing with exposure (Jones & Baxter, 2017; Zhang et al., 2014). However, at elevated levels, exposure becomes toxic and places additional stress on exposed individuals (Daniel et al., 2018; Martin & Barrett, 2002).

Disputably the highest ionizing radiation exposure levels occur in the footprints of nuclear reactor disasters such as those surrounding Three Mile Island, Chernobyl, and Fukushima. Ionizing radiation from natural sources or nuclear accidents has been shown to have significant effects on genotoxicity (Wickliffe et al., 2002), individual morphology (Williams et al., 2001; Møller, 1993), and physiology (Møller et al., 2011), population density (Møller & Mousseau, 2007; Møller & Mousseau, 2009) and community composition (A.P. Møller & Mousseau, 2018). The Chernobyl Exclusion Zone (CEZ) is arguably the most well studied nuclear accident, with diverse studies on wildlife exploring the impacts of environmental radiation exposure, and it provides an ideal model for investigating adaptive response mechanisms associated with radiation exposure. The CEZ has been proposed as the largest ecological sink ever observed (Møller et al., 2012), exhibiting reduced wildlife diversity and abundance (Møller & Mousseau, 2007; Møller & Mousseau, 2018; Romanoskaya, et al. 1998). Additionally, within the CEZ, phenotypic effects such as asymmetry (Møller, 2002), reduced sperm quality (Møller et al., 2014), and small brain size (Møller et al., 2011) have been observed.

However, there have also been arguments made that many studies did not have adequate dosimetry (Hinton et al., 2013) or address potential ecological shifts contributing to the effects observed (Smith, 2008). Moreover, resent research has also reported abundant mammal populations in the CEZ, with no observable impact of radiation exposure on community abundance and distribution (Deryabina et al., 2015; Webster et al., 2016), diversity of aquatic

macroinvertebrates (Murphy, Nagorskaya, & Smith, 2011), or vertebrate scavenger diversity and occurrence (Schlichting et al., 2019). This suggests that there may be two dominating forces at play, the first being the negative impacts of radiation exposure, and the second being the beneficial impacts of removing humans from a landscape. To better understand the dynamics occurring within the CEZ, we need to expand our research from population and community assessments, to address whether these population consist of largely transient individuals and if resident individuals are unhealthy or part of populations that have adapted to the contaminated environments.

Radiation contamination exposure may act like many other stressors in which populations have persisted. As many organisms are predisposed to some radiation from natural sources, the mechanisms underlying acclimation or adaptation to ionizing radiation contaminated landscapes may facilitate more rapid adaptation. Previous studies of radiation induced responses in lab and human models have found dynamic immune responses (Liu, 2003) and a few candidate genes associated with beneficial gene regulation, primarily focusing on genomic instability, DNA repair and oxidative stress genes (O. Kovalchuk et al., 2004; Loree et al., 2006; Raiche, Rodriguez-Juarez, Pogribny, & Kovalchuk, 2004). While studies on wild populations of European wood mice (*Apodemus sylvaticu*s) from uranium mining sites (Lourenço, Pereira, Gonçalves, & Mendo, 2013) and mussels from contaminated sediment near a nuclear facility (Alamri et al., 2012) showed up regulation of specific DNA repair genes. Additionally, studies from the CEZ examining gene specific regulatory patterns in plants (Kovalchuk et al., 2004) and voles (Jernfors et al., 2018) found similar induction of DNA repair gene expression.

The candidate gene approach, however, fails to describe many larger molecular processes and more complex gene interactions critical for adaptive responses to ionizing radiation. Whole

transcriptome studies are better able to describe gene network interactions and inform physiological consequences of exposure. For example, a whole transcriptome study of voles in the CEZ uncovered changes in multiple metabolic and immune pathways (Kesäniemi et al., 2019) and suggests there are cellular and molecular interactions being affected in individuals chronically exposed to radiation.

Here we use a *de novo* RNA sequencing assembly technique to examine whole transcriptome patterns in blood of raccoon dogs (*Nyctereutes procyonoides*) living in the CEZ. Mammals are one of the most radiosensitive taxa (Whicker & Schultz, 1982) and raccoon dogs are an ideal model species because they are longer lived and wider ranging than previously examined species (e.g. voles). Blood is an ideal tissue for examining the impacts of environmental radiation exposure as it is a key player in many immunological responses, as well as an indicator of some of the most radiosensitive tissues in the body (Chaffey & Hellman, 1971). We predicted genes associated with immune response, DNA repair and mitochondrial regulation will be the most affected in raccoon dogs exposed to elevated radiation.

METHODS

Study species

Raccoon dogs were introduced from East Asia to European regions of the Soviet Union in the early 1900s as a result of the fur trade (Lavrov, 1971). In the decades following introduction, this species has expanded its range and become a common canid in Europe (Kowalczyk & Zalewski, 2011; Laurimaa et al., 2016). The average life span of raccoon dogs in Europe is 3-5 years with a maximum of about 8 years (Helle & Kauhala, 1995). Additionally, raccoon dogs are primarily carnivorous, with birds, amphibians, and small mammals making up

the bulk of their diet, however they are opportunistic and will forage on carrion, fruits, and other forms of vegetation. Thus, they are exposed to varying levels of radiation through their diet.

Sample collection

In the fall of 2014, we live trapped raccoon dogs (n = 6) in the largest portion of the CEZ, the Polesye State Radiation Ecological Reserve (PSRER). The CEZ was established after the Chernobyl reactor exploded, releasing over 45,300 kgs of radioactive material into the atmosphere. Much of the radiation settled over western Russia and Europe, with the highest concentrations observed in what is now northern Ukraine and southern Belarus, devastating local communities and ecosystems. Following the disaster, a 4,762 km^2 exclusion zone (CEZ) was established and more than 200,000 people were evacuated from the most contaminated areas. The PSRER serves as the managing entity on the Belarus side of the CEZ and has spatial heterogeneity in radiation contamination distribution (soil contamination levels of 40 – >7000 kBq/m^2), a diverse mammal community (Schlichting et al., 2019; Webster et al., 2016), and lack of human activity in the zone.

We further collaborated with biologists and hunt clubs north of the CEZ, in Belarus, to collect samples (n = 2) from an area with no elevated levels of ionizing radiation. These samples were collected from areas with limited human activity, mixed hardwood and coniferous forests, freshwater lakes and streams, and exhibiting habitat similar to what is found in the CEZ.

Internal radiocesium (Cs-137) activity quantification

We estimated raccoon dog Cs-137 exposure for live-trapped or hunted individuals using home range soil contamination and muscle radioactivity estimates respectively. To estimate exposure for live-trapped individuals within the CEZ, we implemented an area weighted mean of soil contamination technique rather than a single Cs-137 point count to better estimate exposure

likely experienced in a heterogeneously contaminated landscape. To implement this technique, we calculated soil Cs-137 contamination area-weighted means within a 1000 m radius of raccoon dog trap locations. Each trap location was identified with UTM coordinate referenced against geo-referenced CEZ Cs-137 soil contamination maps (Izrael & Bogdevich, 2009; Webster et al., 2016). Radiation exposure rates from hunted individuals were performed according to protocols described earlier (Chapter 3).

RNA sequencing and transcriptome assembly

RNA isolation from whole blood samples was performed using RiboPure Blood Kit (Life Technologies, 2011) where we followed standard manufacturer's protocols. The Georgia Genomics and Bioinformatics Core performed RNA sequencing library preparation and ran all samples across four lanes for sequencing using Illumina NextSeq PE75.

To assess initial read quality we screened all reads with FastQC (S, 2010), performed kmer-based error correction of RNAseq raw reads in Rcorrector (Song & Florea, 2015), and quality controlled while filtering adaptor-containing reads in TrimGalore (Krueger et al., n.d.). Due to lack of a full reference genome assembly for raccoon dogs, trimmed reads were *de novo* assembled with Trinity v2.6.6 (Grabherr et al., 2013). To create a more robust assembly and identify an optimum number of unique genes, we added publicly availably raccoon dog transcriptome data (Du et al., 2017) to our Trinity assembly step. These data were quality controlled in the same manner as described above and only utilized for Trinity assembly. Assembly quality and completeness was assessed by computing E90N50 values and BUSCO v4.0.0 (Seppey, Manni, & Zdobnov, 2019) scores.

Functional annotation of the assembled transcriptome was conducted with the Trinotate suite v3.0.1 (https suite (http://trinotate.github.io/). We first scanned the Trinity transcripts for

coding regions using TransDecoder (Haas et al., 2013) and screened all translations for open reading frames with a minimum length of 100 amino acids. We further characterized the identified peptides based on sequence similarity, using Blast (e-value < 1e-03), against non-redundant Uniref90 and SwissProt databases. We then applied several functional annotation methods to assess the functionality of the transcriptome. We utilized the HMMER approach (Johnson, Eddy, & Portugaly, 2010) and Pfam database (Finn et al., 2014) to identify protein domains, SignalP v5.0 (Almagro Armenteros et al., 2019) to predict the presence of signal peptides, and TMHMM v2.0 (Krogh, Larsson, Von Heijne, & Sonnhammer, 2001) to predict transmembrane regions. Lastly, we created an annotation report by integrating all results into a SQLite database.

Transcript abundance and gene-expression analysis

To perform sample-specific expression analyses we quantified read counts using Salmon (Patro et al., 2017) while referencing our *de novo* assembled raccoon dog target transcripts. Salmon was run with default settings and we examined transcript abundance using hierarchical network method (Oldham, Langfelder, & Horvath, 2012), of which no samples were clear outliers and all samples were retained for downstream analyses (Appendix Figure C1).

To examine patterns of differential expression between the CEZ and the uncontaminated region in northern Belarus we utilized deseq2 (Love, Huber, & Anders, 2014) and edgeR (Robinson, McCarthy, & Smyth, 2009). We normalized read counts by library size using the *cpm* function in edgeR (Robinson et al., 2009) for all downstream analyses and genes were considered expressed if they had ≥ 10.0 counts per million reads and were identified in more than three individuals.

Identification of regulatory modules in raccoon blood transcriptome

To investigate potential gene expression interactions impacted by radiation exposure in the CEZ, we performed weighted gene correlation network analysis (WGCNA) in the WGCNA package in R (Langfelder & Horvath, 2008). To identify outlier samples, we compared mean pairwise correlations. We then used a soft thresholding approach to approximate (Figure C2) a scale free topological network to compare an adjacency matrix (Langfelder & Horvath, 2008). To create a cluster dendrogram we used topological overlap with signed correlations while implementing a minimum cluster size of 20 genes and merging closely correlated modules (R^2 = 0.75, Figure 5.2).

To assess candidate modules associated with the radiation exposure within the CEZ (FDR < 0.05), we applied linear mixed effect models using trap site location and radiation exposure estimates. The 10 most highly connected (top hub) genes were assessed within modules of interest to help describe module gene function (Table 5.2).

RESULTS

De novo transcriptome assembly and annotation

In total 198,823,690 paired-end reads were generated across all eight samples, with a mean quality score of 35.4. In total, we identified 194,823 transcripts (contigs) and 153,362 clusters (genes) in our *de novo* assembly, with a transcript N50 = 1781 bp. The average sample mapping rate to the Trinity assembly was 92.4%. The full assembled transcriptome BUSCO analysis implies a successful assembly with 87.1% complete and 6.7% fragmented genes detected in our assembly. Transcriptome annotation resulted in 62,873 uniproteins from the blastx search against UniProt/SwissProt, represented by near full-length transcripts (>80% alignment coverage).

Variation in blood transcription associated with the CEZ

In total 16,326 genes were considered expressed in the blood transcriptome. Of these, 1,099 genes were differentially expressed in raccoon dogs from the two sampling locations, with 644 genes up-regulated and 455 down-regulated in the CEZ individuals (Figure C3). Expression patterns clearly diverged between sampling locations, with individuals from the CEZ falling out in PCA (Figure 5.1). Of the 40 most differentially expressed genes, 39 were successfully annotated to at least one known functional protein. The most highly differentially expressed genes in the blood transcriptome of raccoon dogs in the CEZ are associated with immune response, cellular transport, and metabolism (Table 5.1).

Identification of regulatory modules in raccoon dog blood transcriptome

We identified seven co-expression modules in the blood transcriptome (Figure 5.2). Expression within two of these modules, the green and brown modules, was significantly associated with raccoon dogs in the CEZ. Both modules exhibited regulatory divergence between raccoon dogs from the CEZ and BLR (F-statistic: 55.56 on 3 and 4 DF, p-value: 0.001), and the green module exhibited a population by radiation interaction ($p < 0.001$). To further assess the biological function of these modules, we assessed the top ten connected genes ('hub' genes) within each module. Of the brown module genes, only one gene was successfully annotated, while all of the green module hub genes were annotated (Table 5.2).

DISCUSSION

Radiation exposure can have detrimental impacts on immune system regulation, genotoxicity and metabolic processes. Gene expression can facilitate individual acclimation and population adaptation to environmental contaminants. Here we describe gene expression patterns indicative of radiation induced changes in raccoon dogs exposed to environmental radiation exposure in the CEZ.

In raccoon dogs, whole transcriptome regulation diverges between individuals from the CEZ and northern Belarus. Genes involved in immune response, cellular ion transport, and cell migration show the most divergence. This is supported by a suite of biomedical studies which describe radiation induced responses in immune, cell migration, metabolic, and DNA repair pathways (e.g. (Kesäniemi et al., 2019; Misra et al., 2006; Su et al., 2012). Immune related genes described in the most highly differentially expressed genes are both up and down regulated. Similar patters have been seen in humans. Atomic bomb survivors exhibit altered T lymphocyte and inflammatory cytokine levels (Kusunoki & Hayashi, 2008), and some inflammatory responses, such as macrophage activity, show stimulation at low radiation exposures (< 0.1 Gy) of radiation exposure and suppression at high exposures (> 2 Gy) (Liu, 2003).

Individual gene expression patterns indicate mechanisms to repair DNA damage and promote cellular homeostasis are being activated. Expression of the three most significantly down regulated genes (ABTB1, CCL15, and CKAP5) is associated with carcinomas in humans (Li, Wu, & Zhang, 2016; Schneider et al., 2017; Wan et al., 2018), potentially signaling gene regulation involved in mitigating oncogenesis. The TLN1 gene was one of the most highly up regulated in raccoon dogs of the CEZ. This gene was also identified as an up-regulated candidate gene under selection in wolves from the CEZ (Chapter 2). This gene's function is associated with

facilitating the connection of cytoskeletal structures to the plasma membrane and release of myeloid leukemia cells into circulation (Badie et al., 2016) as well as metastasis in various cancers (Sakamoto et al., 2010). While this gene is associated with various oncogenic effects in humans, further research is needed to describe its oncogenic correlations in wild canids.

Gene co-expression modules in the raccoon dog blood transcriptome described genes which likely interact in the same functional or regulatory networks. The top hub genes represent highly connected genes within these modules and may be driving expression patterns within these modules. Of the five modules identified in the blood transcriptome, expression within the brown module is associated with site of origin and the green module with both site of origin and radiation exposure. Few genes within the brown module were annotated, however the one gene that was, LORF2, is associated with LINE-1 retro transcription and to our knowledge has not been described in previous radiation research. In the green module, top hub genes are associated with immune response and tumor regulation. We are not yet able to describe potential cumulative biological function for these genes and this is an area of ongoing investigation.

Collectively, these data describe similar genes and networks associated with environmental radiation exposure as described in biomedical studies (O. Kovalchuk et al., 2004; Loree et al., 2006; Raiche et al., 2004). Additionally, similarities in functional groups expressed in wolves (Chapter 2), voles (Kesäniemi et al., 2019), and raccoon dogs, inform genes of interest for further research when examining target genes of selection as well as oncogenic genes which may have accumulated mutations and rendering them inactive. Interestingly we did not find a strong signal of mitochondrial associated gene upregulation as we had expected, perhaps due to adaptive regulatory patterns accumulated over the >30 years of radiation exposure. Further research is needed to confirm these findings by incorporating more robust sample sizes, as well

as addressing more fine scale ecological difference between the two sampling locations which may be additive drivers of the patterns observed here.

In conclusion, our data show clear diverging gene regulatory patterns in pathways with known radiation resistance capabilities (Morgan & Lawrence, 2015; Zhao et al., 2017) and supports the idea that raccoon dogs residing within the CEZ may be adapting to radiation exposure within the zone. Expression patterns described here inform candidate genomic pathways important for acclimation and adaptation to elevated radiation exposure in a wild free ranging species.

REFERENCES

Alamri, O. D., Cundy, A. B., Di, Y., Jha, A. N., & Rotchell, J. M. (2012). Ionizing radiation-induced DNA damage response identified in marine mussels, Mytilus sp. *Environmental Pollution*, *168*, 107–112. https://doi.org/10.1016/j.envpol.2012.04.015

Almagro Armenteros, J. J., Tsirigos, K. D., Sønderby, C. K., Petersen, T. N., Winther, O., Brunak, S., … Nielsen, H. (2019). SignalP 5.0 improves signal peptide predictions using deep neural networks. *Nature Biotechnology*, *37*(4), 420–423. https://doi.org/10.1038/s41587-019-0036-z

Asselman, J., Glaholt, S. P., Smith, Z., Smagghe, G., Janssen, C. R., Colbourne, J. K., … De Schamphelaere, K. A. C. (2012). Functional characterization of four metallothionein genes in Daphnia pulex exposed to environmental stressors. *Aquatic Toxicology*, *110–111*, 54–65. https://doi.org/10.1016/j.aquatox.2011.12.010

Badie, C., Blachowicz, A., Barjaktarovic, Z., Finnon, R., Michaux, A., Sarioglu, H., … Bouffler, S. D. (2016). Transcriptomic and proteomic analysis of mouse radiation-induced acute

myeloid leukaemia (AML). *Oncotarget*, *7*(26), 40461–40480. https://doi.org/10.18632/oncotarget.9626

Bateman, A. (2019). UniProt: A worldwide hub of protein knowledge. *Nucleic Acids Research*, *47*(D1), D506–D515. https://doi.org/10.1093/nar/gky1049

Chaffey, J. T., & Hellman, S. (1971). Differing Responses to Radiation of Murine Bone Marrow Stem Cells in Relation to the Cell Cycle. *Cancer Research*, *31*(11), 1613–1615.

Daniel, S., Nylander, V., Ingerslev, L. R., Zhong, L., Fabre, O., Clifford, B., … Simar, D. (2018). T cell epigenetic remodeling and accelerated epigenetic aging are linked to long-term immune alterations in childhood cancer survivors 11 Medical and Health Sciences 1107 Immunology. *Clinical Epigenetics*, *10*(1), 1–13. https://doi.org/10.1186/s13148-018-0561-5

Deryabina, T. G., Kuchmel, S. V., Nagorskaya, L. L., Hinton, T. G., Beasley, J. C., Lerebours, A., & Smith, J. T. (2015). Long-term census data reveal abundant wildlife populations at Chernobyl. *Current Biology*, *25*(19), R824–R826. https://doi.org/10.1016/j.cub.2015.08.017

Donelson, J. M., Munday, P. L., McCormick, M. I., & Pitcher, C. R. (2012). Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change*, *2*(1), 30–32. https://doi.org/10.1038/nclimate1323

Du, Z., Huang, K., Zhao, J., Song, X., Xing, X., Wu, Q., … Xu, C. (2017). Comparative Transcriptome Analysis of Raccoon Dog Skin to Determine Melanin Content in Hair and Melanin Distribution in Skin. *Sci Rep*, *7*(4899), 40903. https://doi.org/10.1038/srep40903

Williams, D., Nesterovitch, A. I., Tavares, A. F., & Muzzatti, E. G. (2001). Morphological deformities occurring in Belarusian chironomids (Diptera: Chironomidae) subsequent to the Chernobyl nuclear disaster. *Freshwater Biology*, *46*(4), 503–512.

https://doi.org/10.1046/j.1365-2427.2001.00699.x

Finn, R. D., Bateman, A., Clements, J., Coggill, P., Eberhardt, R. Y., Eddy, S. R., … Punta, M. (2014). Pfam: The protein families database. *Nucleic Acids Research*, *42*(D1), 222–230. https://doi.org/10.1093/nar/gkt1223

Flynn, R. W., Love, C. N., Coleman, A., & Lance, S. L. (2019). Variation in metal tolerance associated with population exposure history in Southern toads (Anaxyrus terrestris). *Aquatic Toxicology*, *207*. https://doi.org/10.1016/j.aquatox.2018.12.009

Grabherr, M. G. ., Brian J. Haas, Moran Yassour Joshua Z. Levin, Dawn A. Thompson, Ido Amit, Xian Adiconis, Lin Fan, Raktima Raychowdhury, Qiandong Zeng, Zehua Chen, Evan Mauceli, Nir Hacohen, Andreas Gnirke, Nicholas Rhind, Federica di Palma, Bruce W., N., & Friedman, and A. R. (2013). Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nature Biotechnology*, *29*(7), 644–652. https://doi.org/10.1038/nbt.1883.Trinity

Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Philip, D., Bowden, J., … Regev, A. (2013). De novo transcript sequence recostruction from RNA-Seq: reference generation and analysis with Trinity. In *Nature protocols* (Vol. 8). https://doi.org/10.1038/nprot.2013.084.De

Helle, E., & Kauhala, K. (1995). Reproduction in the Raccoon Dog in Finland Author (s): Eero Helle and Kaarina Kauhala Published by : American Society of Mammalogists Stable URL : http://www.jstor.com/stable/1382597 REFERENCES Linked references are available on JSTOR for this article : *Journal of Mammalogy*, *76*(4), 1036–1046.

Hinton, T. G., Garnier-Laplace, J., Vandenhove, H., Dowdall, M., Adam-Guillermin, C., Alonzo, F., … Vives i Batlle, J. (2013). An invitation to contribute to a strategic research agenda in

radioecology. *Journal of Environmental Radioactivity*, *115*, 73–82. https://doi.org/10.1016/j.jenvrad.2012.07.011

Hoffmann, A. a, & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*(7335), 479–485. https://doi.org/10.1038/nature09670

Izrael, Y., & Bogdevich, I. (2009). *Atlas of current and predicted consequences of the Chernobyl accident on the affected territories of Russia and Belarus*. Minsk, Belarus: Belkartographia.

Jernfors, T., Kesäniemi, J., Lavrinienko, A., Mappes, T., Milinevsky, G., Møller, A. P., … Watts, P. C. (2018). Transcriptional Upregulation of DNA Damage Response Genes in Bank Voles (Myodes glareolus) Inhabiting the Chernobyl Exclusion Zone. *Frontiers in Environmental Science*, *5*(January), 1–8. https://doi.org/10.3389/fenvs.2017.00095

Johnson, L. S., Eddy, S. R., & Portugaly, E. (2010). Hidden Markov model speed heuristic and iterative HMM search procedure. *BMC Bioinformatics*, *11*. https://doi.org/10.1186/1471-2105-11-431

Jones, D. L., & Baxter, B. K. (2017). DNA repair and photoprotection: Mechanisms of overcoming environmental ultraviolet radiation exposure in halophilic archaea. *Frontiers in Microbiology*, *8*(SEP), 1–16. https://doi.org/10.3389/fmicb.2017.01882

Kesäniemi, J., Jernfors, T., Lavrinienko, A., Kivisaari, K., Kiljunen, M., Mappes, T., & Watts, P. C. (2019). Exposure to environmental radionuclides is associated with altered metabolic and immunity pathways in a wild rodent. *Molecular Ecology*, (August), 4620–4635. https://doi.org/10.1111/mec.15241

Kovalchuk, I., Abramov, V., Pogribny, I., Kovalchuk, O., Physiology, S. P., & May, N. (2004). Molecular Aspects of Plant Adaptation to Life in the Chernobyl Zonel [w]. *Plant Physiology*, *135*(1), 357–363. https://doi.org/10.1104/pp.104.040477.stresses

Kovalchuk, O., Burke, P., Besplug, J., Slovack, M., Filkowski, J., & Pogribny, I. (2004). Methylation changes in muscle and liver tissues of male and female mice exposed to acute and chronic low-dose X-ray-irradiation. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, *548*(1–2), 75–84. https://doi.org/10.1016/j.mrfmmm.2003.12.016

Kowalczyk, R., & Zalewski, A. (2011). Adaptation to cold and predation-shelter use by invasive raccoon dogs Nyctereutes procyonoides in Białowieża Primeval Forest (Poland). *European Journal of Wildlife Research*, *57*(1), 133–142. https://doi.org/10.1007/s10344-010-0406-9

Krogh, A., Larsson, B., Von Heijne, G., & Sonnhammer, E. L. L. (2001). Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. *Journal of Molecular Biology*, *305*(3), 567–580. https://doi.org/10.1006/jmbi.2000.4315

Krueger, F., James, F., Ewels, P., & Schuster-Boeckler, B. (n.d.). *Trim Galore*. Retrieved from https://github.com/FelixKrueger/TrimGalore

Kusunoki, Y., & Hayashi, T. (2008). Long-lasting alterations of the immune system by ionizing radiation exposure: implications for disease development among atomic bomb survivors. *International Journal of Radiation Biology*, *84*(1), 1–14. https://doi.org/10.1080/09553000701616106

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*. https://doi.org/10.1186/1471-2105-9-559

Laurimaa, L., Süld, K., Davison, J., Moks, E., Valdmann, H., & Saarma, U. (2016). Alien species and their zoonotic parasites in native and introduced ranges: The raccoon dog example. *Veterinary Parasitology*, *219*, 24–33. https://doi.org/10.1016/j.vetpar.2016.01.020

Lavrov, N. (1971). Results of raccoon dog introductions in different parts of the Soviet Union. *Trudy Kafedry Biologii MGZPI*, *29*, 101–160.

Li, Y., Wu, J., & Zhang, P. (2016). CCL15/CCR1 axis is involved in hepatocellular carcinoma cells migration and invasion. *Tumor Biology*, *37*(4), 4501–4507. https://doi.org/10.1007/s13277-015-4287-0

Life Technologies. (2011). RiboPure™-Blood Kit Protocol (PN 1928M Rev D). *Protocol*.

Liu, S.-Z. (2003). Nonlinear dose-response relationship in the immune system following exposure to ionizing radiation: mechanisms and implications. *Nonlinearity in Biology, Toxicology, Medicine*, *1*(1), 71–92.

Loree, J., Koturbash, I., Kutanzi, K., Baker, M., Pogribny, I., & Kovalchuk, O. (2006). Radiation-induced molecular changes in rat mammary tissue: possible implications for radiation-induced carcinogenesis. *International Journal of Radiation Biology*, *82*(11), 805–815. https://doi.org/10.1080/09553000600960027

Lourenço, J., Mendo, S., & Pereira, R. (2016). Radioactively contaminated areas: Bioindicator species and biomarkers of effect in an early warning scheme for a preliminary risk assessment. *Journal of Hazardous Materials*, *317*, 503–542. https://doi.org/10.1016/j.jhazmat.2016.06.020

Lourenço, J., Pereira, R., Gonçalves, F., & Mendo, S. (2013). Metal bioaccumulation, genotoxicity and gene expression in the European wood mouse (Apodemus sylvaticus) inhabiting an abandoned uranium mining area. *Science of the Total Environment*, *443*, 673–680. https://doi.org/10.1016/j.scitotenv.2012.10.105

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 1–21.

https://doi.org/10.1186/s13059-014-0550-8

Martin, K. R., & Barrett, J. C. (2002). Reactive oxygen species as double-edged swords in cellular processes: Low-dose cell signaling versus high-dose toxicity. *Human and Experimental Toxicology*, *21*(2), 71–75. https://doi.org/10.1191/0960327102ht213oa

Misra, H. S., Khairnar, N. P., Kota, S., Shrivastava, S., Joshi, V. P., & Apte, S. K. (2006). An exonuclease I-sensitive DNA repair pathway in Deinococcus radiodurans: A major determinant of radiation resistance. *Molecular Microbiology*, *59*(4), 1308–1316. https://doi.org/10.1111/j.1365-2958.2005.05005.x

Møller, A. P. (1993). Morphology and sexual selection in the barn swallow Hirundo rustica in Chernobyl, Ukraine. *Proceedings of the Royal Society B: Biological Sciences*, *252*(1333), 51–57. https://doi.org/10.1098/rspb.1993.0045

Møller, A. P., & Mousseau, T. A. (2007). Species richness and abundance of forest birds in relation to radiation at Chernobyl. *Biology Letters*, *3*(5), 483–486. https://doi.org/10.1098/rsbl.2007.0226

Møller, A.P., & Mousseau, T. A. (2018). Reduced colonization by soil invertebrates to irradiated decomposing wood in Chernobyl. *Science of The Total Environment*, *645*, 773–779. https://doi.org/10.1016/j.scitotenv.2018.07.195

Møller, A P, Bonisoli-Alquati, A., Rudolfsen, G., & Mousseau, T. A. (2012). Elevated mortality among birds in Chernobyl as judged from skewed age and sex ratios. *PLoS ONE*, *7*(4), 1–8. https://doi.org/10.1371/journal.pone.0035223

Møller, A. P. (2002). Developmental instability and sexual selection in stag beetles from Chernobyl and a control area. *Ethology*, *108*(3), 193–204. https://doi.org/10.1046/j.1439-0310.2002.00758.x

Møller, A. P., Bonisoli-Alquati, A., Mousseau, T. A., & Rudolfsen, G. (2014). Aspermy, sperm quality and radiation in chernobyl birds. *PLoS ONE*, *9*(6). https://doi.org/10.1371/journal.pone.0100296

Møller, A. P., Bonisoli-Alquati, A., Rudolfsen, G., & Mousseau, T. A. (2011). Chernobyl birds have smaller brains. *PLoS ONE*, *6*(2). https://doi.org/10.1371/journal.pone.0016862

Møller, A. P., & Mousseau, T. a. (2009). Reduced abundance of insects and spiders linked to radiation at Chernobyl 20 years after the accident. *Biology Letters*, *5*(3), 356–359. https://doi.org/10.1098/rsbl.2008.0778

Morgan, M. A., & Lawrence, T. S. (2015). Molecular pathways: Overcoming radiation resistance by targeting DNA damage response pathways. *Clinical Cancer Research*, *21*(13), 2898–2904. https://doi.org/10.1158/1078-0432.CCR-13-3229

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

Oldham, M. C., Langfelder, P., & Horvath, S. (2012). Network methods for describing sample relationships in genomic datasets: application to Huntington's disease. *BMC Systems Biology*, *6*. https://doi.org/10.1186/1752-0509-6-63

Otto, S. P. (2018). Adaptation, speciation and extinction in the Anthropocene. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1891). https://doi.org/10.1098/rspb.2018.2047

Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change. *Nature*, *421*, 37–42. https://doi.org/10.1038/nature01286

Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., & Kingsford, C. (2017). Salmon provides fast

and bias-aware quantification of transcript expression. *Nature Methods*, *14*(4), 417–419. https://doi.org/10.1038/nmeth.4197

Raiche, J., Rodriguez-Juarez, R., Pogribny, I., & Kovalchuk, O. (2004). Sex- and tissue-specific expression of maintenance and de novo DNA methyltransferases upon low dose X-irradiation in mice. *Biochemical and Biophysical Research Communications*, *325*(1), 39–47. https://doi.org/10.1016/j.bbrc.2004.10.002

Reid, N. M., Proestou, D. A., Clark, B. W., Warren, W. C., Colbourne, J. K., Shaw, J. R., … Whitehead, A. (2016). The genomic landscape of rapid repeated evolutionary adaptation to toxic pollution in wild fish. *Science*, *354*(6317), 1305–1308. https://doi.org/10.1126/science.aah4993

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2009). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, *26*(1), 139–140. https://doi.org/10.1093/bioinformatics/btp616

Romanoskaya, V. A., I.G., S., R.V., R., & N.A., C. (1998). Effect of radioactive contamination on soil bacteria in the 10-km zone around the Chernobyl nuclear power plant. *Microbiology*, *67*(2), 226–231.

Andrews, S. (2010). *FastQC: a quality control tool for high throughput sequence data*. Retrieved from http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Sakamoto, S., McCann, R. O., Dhir, R., & Kyprianou, N. (2010). Talin1 promotes tumor invasion and metastasis via focal adhesion signaling and anoikis resistance. *Cancer Research*, *70*(5), 1885–1895. https://doi.org/10.1158/0008-5472.CAN-09-2833

Schlichting, P. E., Love, C. N., Webster, S. C., & Beasley, J. C. (2019). Efficiency and composition of vertebrate scavengers at the land-water interface in the Chernobyl Exclusion

Zone. *Food Webs*, *18*. https://doi.org/10.1016/j.fooweb.2018.e00107

Schneider, M., Christopoulos, P., Muley, T., Warth, A., Klingmueller, U., Thomas, M., … Meister, M. (2017). AURKA_DLGAP5_TPX2_KIF11_and_CKAP: Five specific mitosis-associated genes correlate with poor prognosis for non-small cell lung cancer pateints. *International Journal of Oncology*, *50*, 365–372.

Seppey, M., Manni, M., & Zdobnov, E. M. (2019). *Gene Prediction Methods and Protocols Methods in Molecular Biology 1962* (M. Kollmar, Ed.). Retrieved from http://www.springer.com/series/7651

Shaw, J. R., Hampton, T. H., King, B. L., Whitehead, a., Galvez, F., Gross, R. H., … Stanton, B. a. (2014). Natural Selection Canalizes Expression Variation of Environmentally Induced Plasticity-Enabling Genes. *Molecular Biology and Evolution*, *31*(11), 3002–3015. https://doi.org/10.1093/molbev/msu241

Shaw, Joseph R., Colbourne, J. K., Glaholt, S. P., Turner, E., Folt, C. L., & Chen, C. Y. (2019). Dynamics of Camium Acclimation in Daphnia pulex: Linking Fitness Costs, Cross-Tolerance, an Hyper-Inuction of Metallothionein. *Environmental Science and Technology*, *53*(24), 14670–14678. https://doi.org/10.1021/acs.est.9b05006

Smith, J. T. (2008). Is Chernobyl radiation really causing negative individual and population-level effects on barn swallows? *Biology Letters*, *4*(1), 63–64; discussion 65-6. https://doi.org/10.1098/rsbl.2007.0430

Song, L., & Florea, L. (2015). Rcorrector: Efficient and accurate error correction for Illumina RNA-seq reads. *GigaScience*, *4*(1), 1–8. https://doi.org/10.1186/s13742-015-0089-y

Šrut, M., Drechsel, V., & Höckner, M. (2017). Low levels of Cd induce persisting epigenetic modifications and acclimation mechanisms in the earthworm Lumbricus terrestris. *PLoS*

ONE, *12*(4), 1–18. https://doi.org/10.1371/journal.pone.0176047

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science (New York, N.Y.)*, *306*(October), 1783–1786. https://doi.org/10.1126/science.1103538

Su, W. H., Chuang, P. C., Huang, E. Y., & Yang, K. D. (2012). Radiation-induced increase in cell migration and metastatic potential of cervical cancer cells operates via the K-ras pathway. *American Journal of Pathology*, *180*(2), 862–871. https://doi.org/10.1016/j.ajpath.2011.10.018

Subramanian, M. (2019). Humans versus Earth: the quest to define the Anthropocene. *Nature*, *572*(7768), 168–170. https://doi.org/10.1038/d41586-019-02381-2

Wan, Q., Tang, J., Han, Y., & Wang, D. (2018). Co-expression modules construction by WGCNA and identify potential prognostic markers of uveal melanoma. *Experimental Eye Research*, *166*(October 2017), 13–20. https://doi.org/10.1016/j.exer.2017.10.007

Waterhouse, M. D., Erb, L. P., Beever, E. A., & Russello, M. A. (2018). Adaptive population divergence and directional gene flow across steep elevational gradients in a climate-sensitive mammal. *Molecular Ecology*, (March), 2512–2528. https://doi.org/10.1111/mec.14701

Webster, S. C., Byrne, M. E., Lance, S. L., Love, C. N., Hinton, W. G., Shamovich, D., & Beasley, J. C. (2016). Where the wild things are: influence of radiation on the distribution of four mammalian species within the Chernobyl Exclusion Zone. *Fronteirs in Ecology and the Environment*, *14*(4), 185–190.

Whitehead, A., Roach, J. L., Zhang, S., & Galvez, F. (2011). Genomic mechanisms of evolved

physiological plasticity in killifish distributed along an environmental salinity gradient. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(15), 6193–6198. https://doi.org/10.1073/pnas.1017542108

Wickliffe, J. K., Chesser, R. K., Rodgers, B. E., & Baker, R. J. (2002). Assessing the genotoxicity of chronic environmental irradiation by using mitochondrial DNA heteroplasmy in the bank vole (Clethrionomys glareolus) at Chornobyl, Ukraine. *Environmental Toxicology and Chemistry / SETAC*, *21*(6), 1249–1254. https://doi.org/10.1002/etc.5620210619

Williams, S. E., Shoo, L. P., Isaac, J. L., Hoffmann, A. A., & Langham, G. (2008). Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biology*, *6*(12). https://doi.org/10.1371/journal.pbio.0060325

Zhang, Y., Dood, J., Beckstead, A. A., Li, X. B., Nguyen, K. V., Burrows, C. J., … Kohler, B. (2014). Efficient UV-induced charge separation and recombination in an 8-oxoguaninecontaining dinucleotide. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(32), 11612–11617. https://doi.org/10.1073/pnas.1404411111

Zhao, H., Ning, S., Nolley, R., Scicinski, J., Oronsky, B., Knox, S. J., & Peehl, D. M. (2017). The immunomodulatory anticancer agent, RRx-001, induces an interferon response through epigenetic induction of viral mimicry. *Clinical Epigenetics*, *9*(1), 1–11. https://doi.org/10.1186/s13148-017-0312-z

TABLES

Table 5.1. Most highly differentially expressed genes (FDR corrected p-values) in raccoon dog blood transcriptome. Positive fold change (logFC) values indicate upregulation in CEZ individuals. Gene descriptions as directly described by the UniProt (Bateman, 2019) database.

Up Regulated Genes				
Gene	**logFC**	**FDR**	**Protein**	**Function/Description**
DEMA	11.7318	0.0000	Envelope glycoprotein gp160	Oligomerizes in the host endoplasmic reticulum into predominantly trimers. In a second time, gp160 transits in the host Golgi, where glycosylation is completed
EZRI	11.4180	0.0064	Ezrin	Probably involved in connections of major cytoskeletal structures to the plasma membrane.
DEMA	11.2574	0.0000	Envelope glycoprotein gp160	Oligomerizes in the host endoplasmic reticulum into predominantly trimers. In a second time, gp160 transits in the host Golgi, where glycosylation is completed
PANK3	11.1330	0.0317	Pantothenate kinase 3	Plays a role in the physiological regulation of coenzyme A (CoA) levels
NCOA4	10.9858	0.0000	Nuclear receptor coactivator 4	Enhances the androgen receptor transcriptional activity in prostate cancer cells.
DDX5	10.1678	0.0000	DEAD-box helicase 5	nvolved in the alternative regulation of pre-mRNA splicing; its RNA helicase activity is necessary for increasing tau exon 10 inclusion and occurs in a RBM4-dependent manner.
PKN1	10.1431	0.0000	Protein kinase N1	PKC-related serine/threonine-protein kinase involved in various processes such as regulation of the intermediate filaments of the actin cytoskeleton, cell migration, tumor cell invasion and transcription regulation.

AQP9	10.0916	0.0094	aquaporin 9	Forms a water channel with a broad specificity
RNF10	10.0864	0.0447	ring finger protein 10	Transcriptional factor involved in the regulation of MAG (Myelin-associated glycoprotein) expression
TBB1	10.0066	0.0147	SH2 domain-containing protein 1A	Cytoplasmic adapter regulating receptors of the signaling lymphocytic activation molecule (SLAM) family such as SLAMF1, CD244, LY9, CD84, SLAMF6 and SLAMF7
ANTR2	9.9388	0.0001	Anthrax toxin receptor 2	Necessary for cellular interactions with laminin and the extracellular matrix
MGA	9.9324	0.0012	MAX dimerization protein MGA	Functions as a dual-specificity transcription factor, regulating the expression of both MAX-network and T-box family target genes
A19	9.8876	0.0372	DLA class I histocompatibility antigen, A9/A9 alpha chain	Transporter that mediates resorption of neutral amino acids across the apical membrane of renal and intestinal epithelial cells
SEM4D	9.7692	0.0008	Semaphorin-4D	Cell surface receptor for PLXNB1 and PLXNB2 that plays an important role in cell-cell signaling
NQO1	9.7539	0.0146	NAD(P)H quinone dehydrogenase 1	The enzyme apparently serves as a quinone reductase in connection with conjugation reactions of hydroquinons involved in detoxification pathways as well as in biosynthetic processes such as the vitamin K-dependent gamma-carboxylation of glutamate residues in prothrombin synthesis.
PRAM	9.6909	0.0000	PML-RARA-regulated adapter molecule 1	May be involved in myeloid differentiation.
PP6R3	9.6131	0.0000	Serine/threonine-protein phosphatase 6 regulatory subunit 3	Transporter that mediates resorption of neutral amino acids across the apical membrane of renal and intestinal epithelial cells

TNR1B	9.5746	0.0048	Tumor necrosis factor receptor superfamily member 1B	Receptor with high affinity for TNFSF2/TNF-alpha and approximately 5-fold lower affinity for homotrimeric TNFSF1/lymphotoxin-alpha.
ITB7	9.5272	0.0009	Integrin beta-7	adhesion molecule that mediates lymphocyte migration and homing to gut-associated lymphoid tissue
TLN1	9.5231	0.0016	talin 1	Probably involved in connections of major cytoskeletal structures to the plasma membrane.
SOS2	9.5087	0.0000	SOS Ras/Rho guanine nucleotide exchange factor 2	Promotes the exchange of Ras-bound GDP by GTP
R3HD4	9.4480	0.0002	R3H domain-containing protein 4	nucleic acid binding
MED14	9.3218	0.0001	mediator complex subunit 14	Component of the Mediator complex, a coactivator involved in the regulated transcription of nearly all RNA polymerase II-dependent genes
XPO6	9.2033	0.0231	Exportin-6	Mediates the nuclear export of actin and profilin-actin complexes in somatic cells.
FMNL1	9.0576	0.0003	formin like 1	May play a role in the control of cell motility and survival of macrophages

Down Regulated Genes				
Gene	**logFC**	**FDR**	**Protein**	**Description**
ABTB1	-8.4449	0.0000	Ankyrin repeat and BTB domain containing 1	May act as a mediator of the PTEN growth-suppressive signaling pathway.
CCL15	-9.2169	0.0000	C-C motif chemokine ligand 15	Chemotactic factor that attracts T-cells and monocytes, but not neutrophils, eosinophils, or B-cells.
CKAP5	-9.4566	0.0001	Cytoskeleton associated protein 5	Binds to the plus end of microtubules and regulates microtubule dynamics and microtubule organization.

CMPK2	-8.9982	0.0003	Cytidine/uridine monophosphate kinase 2	May participate in dUTP and dCTP synthesis in mitochondria.
CPAF3	-10.0951	0.0000	Flp pilus assembly ATPase CpaF	hydrolase activity
GBB2	-9.2766	0.0000	Guanine nucleotide-binding protein subunit beta-2	Mediates the voltage-dependent sodium ion permeability of excitable membranes.
I10R1	-8.7027	0.0004	Interleukin 10 Receptor Subunit Alpha	Cell surface receptor for the cytokine IL10 that participates in IL10-mediated anti-inflammatory functions, limiting excessive tissue disruption caused by inflammation.
KLHL8	-8.3969	0.0000	Kelch like family member 8	Substrate-specific adapter of a BCR (BTB-CUL3-RBX1) E3 ubiquitin ligase complex required for The BCR(KLHL8) ubiquitin ligase complex mediates ubiquitination and degradation of RAPSN.
LDHA	-9.9023	0.0000	Lactate dehydrogenase A	This protein is involved in step 1 of the subpathway that synthesizes (S)-lactate from pyruvate.
LYAM1	-11.2542	0.0000	L-selectin	Calcium-dependent lectin that mediates cell adhesion by binding to glycoproteins on neighboring cells
MMP8	-12.1707	0.0000	Matrix metallopeptidase 8	Can degrade fibrillar type I, II, and III collagens.
MMP8	-8.7990	0.0002	Neutrophil collagenase	Can degrade fibrillar type I, II, and III collagens.
NEK6	-9.3777	0.0001	NIMA related kinase 6	Protein kinase which plays an important role in mitotic cell cycle progression
PCNA	-8.3798	0.0000	PCNA-associated factor	PCNA-binding protein that acts as a regulator of DNA repair during DNA replication.

RASF5	-8.3632	0.0000	Ras association domain-containing protein 5	Potential tumor suppressor.
RL40	-8.9047	0.0000	Ubiquitin-60S ribosomal protein L40	Lys-6-linked may be involved in DNA repair
SC24A	-9.9015	0.0000	Promotes the formation of transport vesicles from the endoplasmic reticulum	promotes the formation of transport vesicles from the endoplasmic reticulum
SRRT	-8.8267	0.0000	Serrate RNA effector molecule homolog	Acts as a mediator between the cap-binding complex (CBC) and the primary microRNAs (miRNAs) processing machinery during cell proliferation.

Table 5.2. Top hub genes in co-expression modules associated with population and radiation exposure. Gene, protein, and functional descriptions (Bateman, 2019).

Brown Module		
Gene	**Protein**	**Function/Description**
LORF2	LINE-1 retrotransposable element ORF2 protein	Has a reverse transcriptase activity required for target-primed reverse transcription of the LINE-1 element mRNA, a crucial step in LINE-1 retrotransposition.

Green Module		
Gene	**Protein**	**Function/Description**
RGS1	Regulator of G-protein signaling 1	Regulates G protein-coupled receptor signaling cascades, including signaling downstream of the N-formylpeptide chemoattractant receptors and leukotriene receptors
NR4A3	Nuclear receptor subfamily 4 group A member 3	Transcriptional activator that binds to regulatory elements in promoter regions in a cell- and response element (target)-specific manner
DGKD	Diacylglycerol kinase delta	May function as signaling molecule. Isoform 2 may be involved in cell growth and tumorigenesis.
ACE	Angiotensin-converting enzyme 2	Essential counter-regulatory carboxypeptidase of the renin-angiotensin hormone system that is a critical regulator of blood volume, systemic vascular resistance, and thus cardiovascular homeostasis

ISG15	Ubiquitin-like protein ISG15	Ubiquitin-like protein which plays a key role in the innate immune response to viral infection either via its conjugation to a target protein (ISGylation) or via its action as a free or unconjugated protein.
TP4A1	Protein tyrosine phosphatase type IVA 1	Protein tyrosine phosphatase which stimulates progression from G1 into S phase during mitosis.
LORF1	LINE-1 retrotransposable element ORF1 protein	Nucleic acid-binding protein which is essential for retrotransposition of LINE-1 elements in the genome.
NR4A1	Nuclear receptor subfamily 4 group A member 1	Orphan nuclear receptor.
MK14	Cytochrome c oxidase subunit 1	Component of the cytochrome c oxidase, the last enzyme in the mitochondrial electron transport chain which drives oxidative phosphorylation.
MMP8	Neutrophil collagenase	Can degrade fibrillar type I, II, and III collagens.

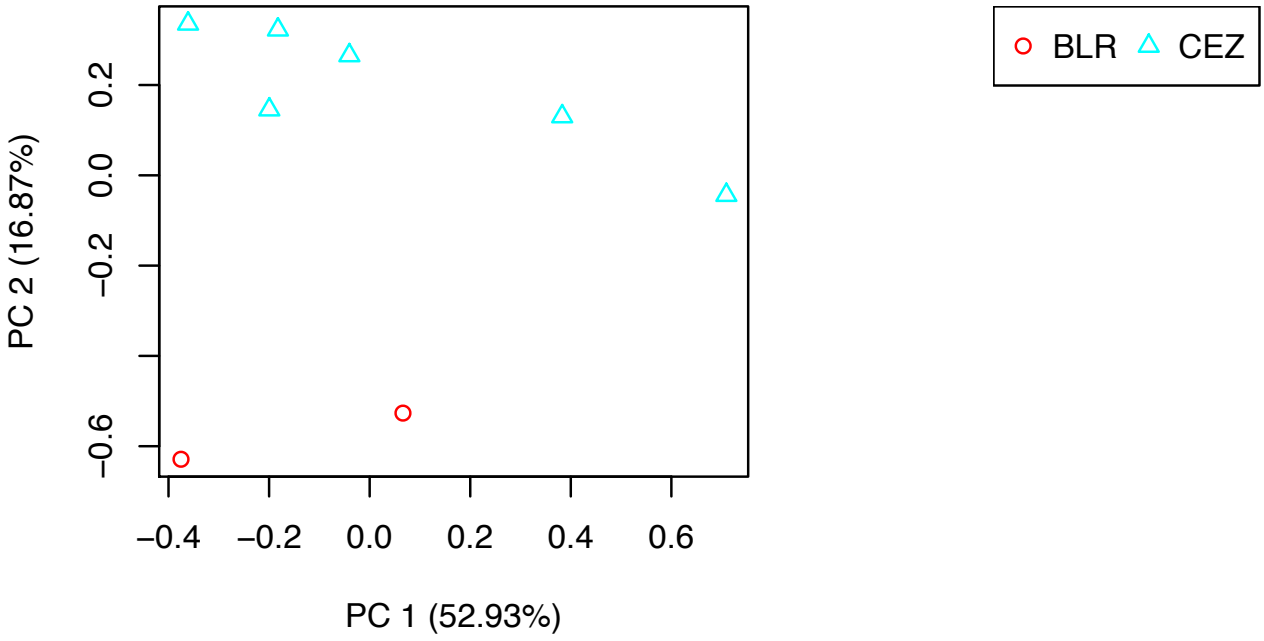

Figure 5.1 Principle components analysis of global transcription patterns in raccoon dogs from northern Belarus (BLR) and the Chernobyl Exclusion Zone (CEZ).

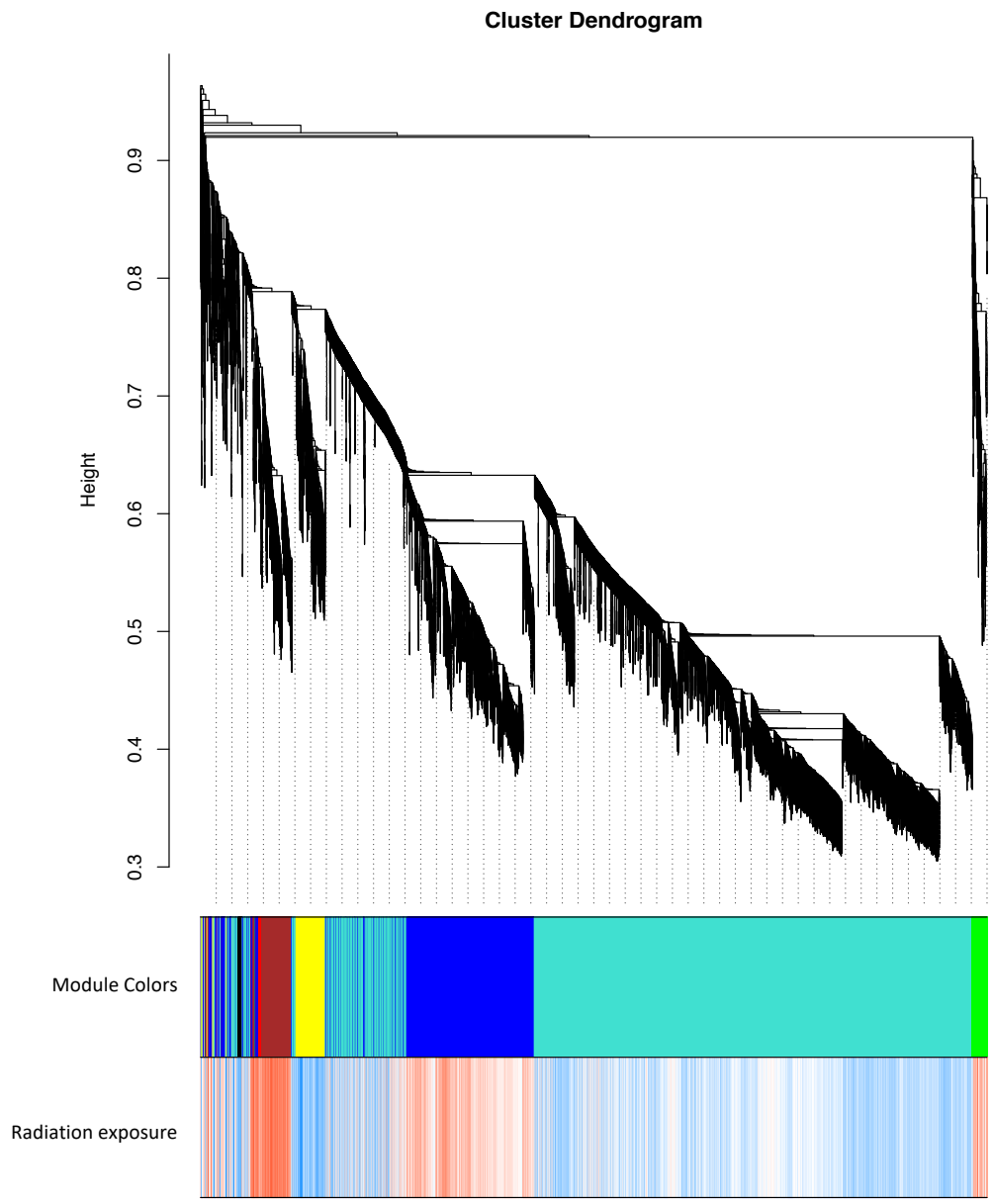

Figure 5.2. Cluster dendrogram of correlated gene expression patterns within the raccoon dog blood transcriptome. Branches represent individual genes while module colors identify groups of highly correlated genes. Radiation exposure shows correlation between each gene and individual Cs-137 exposure variation, red specifies a positive correlation.

CHAPTER 6

CONCLUSION

Wildlife are increasingly inhabiting anthropogenically altered habitats and ecosystems (Otto, 2018; Subramanian, 2019), driving some populations to extinction, while others are able to persist in these novel and stressful environments (Otto, 2018; Parmesan & Yohe, 2003; Stuart et al., 2004; Waterhouse et al., 2018). Biological impoverishment, invasive species, habitat fragmentation, climate change, and toxification have worked collectively to degrade ecosystems and impact population health and persistence. Environmental contamination from industrial practices is a leading way that humans impact the environment, and ionizing radiation contamination as a result of nuclear power rector accidents is a unique model system to explore how wildlife respond to such dramatically affected ecosystems. These systems not only provide an opportunity to examine molecular mechanisms of adaptation to genotoxic substances, but provide a model for exploring potentially plastic and adaptive responses to elevated levels of a stressor which occurs naturally at low levels in forms such as UV radiation and radon (E Stranden, 1979). In this dissertation I utilized genomic techniques and two canid species, gray wolf (*Canis lupus*) and raccoon dog (*Nyctereutes procyonoides*), from the radiation contaminated landscape of the Chernobyl Exclusion Zone (CEZ) to create a model system for examining long-term impacts of elevated radiation exposure. The first goal of my dissertation was to examine genomic and gene regulatory patterns of acclimation and adaptation to elucidate molecular mechanisms underlying population persistence in a habitat highly contaminated with radiation. Second, I explored parasite diversity and prevalence to help assess host health and illuminate

potentially ecologically relevant host-parasite interactions. Lastly, I assessed whether endogenous retroviral element transcription, known to impact immune response, gene regulation, and oncogenesis, (Dai et al., 2018; Morris et al., 2019; Zhao et al., 2017) show signs of radiation-induced activation.

In my first three research chapters I examined genomic signatures of adaptation, regulatory divergence, and health in wolves from the CEZ. In addition to examining population structure and gene expression patters, I also assessed endogenous retroviral immune pathway transcription with relation to elevated radiation exposure and internal radiocesium activity, and micro- and macroparasite diversity and prevalence. In Chapter 2 I found that wolves within the CEZ show distinct population structure from wolves in the north of Belarus that experience background levels of radiation. These finding uniquely describe population differentiation across the CEZ boundary, potentially suggesting contamination within the CEZ is acting as a selective pressure and/or a barrier to gene flow. Additionally, gene expression patterns in blood clearly differ in wolves exposed to radiation within the CEZ, and individual radiocesium activity correlates with immune and oncogenic response pathways. Gene co-expression modules from blood transcriptomes identify immune response genes associated with viral infections as driving some of the primary gene regulation differences correlated with internal radiocesium activity. Furthermore, I identified four proposed putative genes under selection in wolves from the CEZ. These genes were up regulated and are associated with pathways including platelet activation, Human T-cell leukemia virus 1 infection, Rap1 signaling pathway, and focal adhesion (Palmisano, Priami, & Tech, 2013). These data in, addition to the single gene and gene module regulation, also identify genes associated with oncogenesis in humans (Godoy et al., 2013; Singel et al., 2013; Xia et al., 2018) and suggest oncogenesis may be a driving selective force on

individuals inhabiting the CEZ. This work is supported by research in the biomedical field which has a long history of describing radiation induced sarcomas (Brenner et al., 2003), yet this is the first study to elucidate such clear correlations on individual exposure and genomic signals of oncogenesis. This work leads naturally into further examining transcription regulation such as DNA methylation patterns, gene network connectivity shifts, and gene specific mutation rates to further explore genes imperative to adapting to a stressor such as ionizing radiation.

In Chapters 3 and 4, I concentrated on how radiation may impact health of CEZ residents by examining micro- and macroparasite patterns, and endogenous retroviral element activity in radiation exposed individuals. In Chapter 3 I explore parasite prevalence and diversity in both raccoon dogs and wolves. Here I described varying patterns of gastrointestinal parasites, seroprevalence and qPCR identification of microparasites between hosts and across varying radiation exposures. I describe parasites which are both directly and indirectly transmitted and have shown varying degrees of radiosensitivity in lab or human studies (e.g Mezhir et al., 2005; Nansen, Christensen, & Frandsen, 1976). Of the many complex patterns of infection identified, few significantly correlated with individual radiation exposure estimates. This suggests that studies identifying detrimental effects of radiation in parasite and host in isolation or lab studies is not sufficient for successfully describing the complexity of host-parasite-environment interactions radiation contaminated habitats such as this. Interestingly I did find evidence of two viruses, Herpesvirus and canine parvovirus, correlating with internal radiation activity in wolves. These data build off of human models which find increased Herpesvirus activity in individuals exposed to radiation (Mezhir et al., 2005). It has also been proposed that viruses may evolve at a rate which allows them to become radio-resistant (Whicker & Schultz, 1982). My findings in this chapter again suggest virus-radiation interactions are significant and may have serious

implications on reproduction and population dynamics and requires further examination. Collectively, these findings and those viral infection response pathways described in Chapter 1 suggest interactions of increasing viral infections or susceptibility to some viral infection in wolves with radiation exposure, contrary to what has been seen in voles from the CEZ (Kesäniemi et al., 2019). These dynamics warrant closer examination, including examining immunocompetence in canids from the region and viral sequence and virulence studies.

In Chapter 4 I further examined potential viral element interactions with elevated radiation exposure by examining endogenous retroviral (ERV) transcription rates in wolves from inside and outside the CEZ. Here I described clear differences in the number of ERV elements activated with radiation exposure and increased expression correlating with internal radiocesium activity in wolves from inside the CEZ. ERV expression is associated with neurological and oncogenic disease (Morris et al., 2019; Slokar & Hasler, 2016). Building off of this, I explored gene expression patterns in pathways associated with carcinomas and found significant correlations between these expression patterns and ERV expression. I additionally explored immune response pathways and found ERV expression to be correlated with these patterns as well. These data further suggest that oncogenesis – and possible oncogenic mitigation - may play important roles in the selective pressures placed on residents of radiation contaminated environments. This study is purely correlational however, and further work is needed to explore proteomic and physiological consequences of the ERV transcription I described in this chapter.

In my first three research chapters, I observed significant genomic and viral interactions with radiation exposure in wolves, suggesting important genomic and health effects of elevated radiation exposure in wolves. To further explore the impacts of living in an environment with elevated ionizing radiation levels, I examined gene expression patterns in a sympatric canid

species, the raccoon dog, in Chapter 5. In this chapter I conducted a *de novo* assembly to explore gene expression patterns in this canid. Raccoon dogs differ from wolves in various natural history traits and spatial use patterns (Kauhala, Holmala, & Schregel, 2007), and provide an excellent comparison species to begin to encompass some of the variation observed across individuals and species necessary for better understanding ecologically explicit effects of radiation contamination (Rhodes et al., 2020). In this chapter I described raccoon dog global gene expression patterns which diverge between individuals from the CEZ and northern Belarus. In addition to individual gene expression patterns I described two of the five coexpression modules identified in the blood transcriptome and associated with site of origin, and in one case with radiation exposure. The most highly divergent expression patterns between exposed and unexposed individuals similarly fall within biological functions such as immune response, cellular transport, and RNA binding (Bateman, 2019). Interestingly, I found that one of the genes isolated in wolves as a candidate for selection, and associated with oncogenesis in humans, was also one of the most highly upregulated genes in raccoon dogs. With the help of this new transcriptome, further research can delve into the Gene Ontology of hub genes of interest. Next steps also point towards exploring gene network and slice site variation across radiation exposures, and eventually across species. Additionally, once genome assembly and annotation is refined, it will be beneficial to examine patterns of sequence variation and identify candidate genes under selection in the raccoon dogs, similarly to that done in wolves.

In addition to the factors taken into account above, we were not able to encompass all ecological influences which may differ between our two sampling locations and impact the endpoints highlighted in this dissertation. Further research is needed to more thoroughly describe contemporary differences in population densities, food resources, forest composition, and species

competition for potentially limited resources. Given the initial devastation of the forests surrounding the Chernobyl reactor immediately after the explosion, it is possible the reestablishment of this region, along with the lack of human inhabitants, may confer contemporary ecosystem differences not yet documented. Here I attempted to account for these differences by establishing robust individual level radiation exposure estimates and examining patterns of transcription and parasitism with relation to individual exposure estimates.

Collectively, my dissertation demonstrates genomic and transcriptomic signatures of selection to the radiation contaminated landscape of the CEZ and highlights interactive effects of virus infection, transcription, and immune system genes. This study establishes an ideal model system for examining health implications of elevated radiation exposure in wildlife that can also inform human health. Canid species are sentinel species and are frequently utilized as a model for human health (Aguirre, 2009; Ostrander, Dreger, & Evans, 2019). Additionally, by incorporating multiple focal and parasite species responses to varying radiation exposures *in situ*, this research provides vital details for informing population persistence models, risk assessment protocols, and conservation efforts in highly contaminated environments.

REFERENCES

Aguirre, A. A. (2009). Wild canids as sentinels of ecological health: A conservation medicine perspective. *Parasites and Vectors*, *2*(SUPPL.1), 1–8. https://doi.org/10.1186/1756-3305-2-S1-S7

Bateman, A. (2019). UniProt: A worldwide hub of protein knowledge. *Nucleic Acids Research*, *47*(D1), D506–D515. https://doi.org/10.1093/nar/gky1049

Brenner, D. J., Doll, R., Goodhead, D. T., Hall, E. J., Land, C. E., Little, J. B., … Zaider, M.

(2003). Cancer risks attributable to low doses of ionizing radiation: assessing what we really know. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(24), 13761–13766. https://doi.org/10.1073/pnas.2235592100

Dai, L., Del Valle, L., Miley, W., Whitby, D., Ochoa, A. C., Flemington, E. K., & Qin, Z. (2018). Transactivation of human endogenous retrovirus K (HERV-K) by KSHV promotes Kaposi's sarcoma development. *Oncogene*, *37*(33), 4534–4545. https://doi.org/10.1038/s41388-018-0282-4

Stranden, E. (1979). *Population Doses From Naturally Occuring Radiaion*.

Godoy, P. R. D. V., Mello, S. S., Magalhães, D. A. R., Donaires, F. S., Nicolucci, P., Donadi, E. A., … Sakamoto-Hojo, E. T. (2013). Ionizing radiation-induced gene expression changes in TP53 proficient and deficient glioblastoma cell lines. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *756*(1–2), 46–55. https://doi.org/10.1016/j.mrgentox.2013.06.010

Kauhala, K., Holmala, K., & Schregel, J. (2007). Seasonal activity patterns and movements of the raccoon dog, a vector of diseases and parasites, in southern Finland. *Mammalian Biology*, *72*(6), 342–353. https://doi.org/10.1016/j.mambio.2006.10.006

Kesäniemi, J., Lavrinienko, A., Tukalenko, E., Mappes, T., Watts, P. C., & Jurvansuu, J. (2019). Infection load and prevalence of novel viruses identified from the bank vole do not associate with exposure to environmental radioactivity. *Viruses*, *12*(1). https://doi.org/10.3390/v12010044

Mezhir, J. J., Advani, S. J., Smith, K. D., Darga, T. E., Poon, A. P. W., Schmidt, H., … Weichselbaum, R. R. (2005). Ionizing radiation activates late herpes simplex virus 1 promoters via the p38 pathway in tumors treated with oncolytic viruses. *Cancer Research*,

65(20), 9479–9484. https://doi.org/10.1158/0008-5472.CAN-05-1927

Morris, G., Maes, M., Murdjeva, M., & Puri, B. K. (2019). Do Human Endogenous Retroviruses Contribute to Multiple Sclerosis, and if So, How? *Molecular Neurobiology*, *56*(4), 2590–2605. https://doi.org/10.1007/s12035-018-1255-x

Nansen, P., Christensen, N., & Frandsen, F. (1976). A technique for in vivo labelling of Fascioa hepatica miracida with radioselenium. *Zeitschrift Fur Parasitenkunde*, *49*(1), 73–78.

Ostrander, E. A., Dreger, D. L., & Evans, J. M. (2019). Canine Cancer Genomics: Lessons for Canine and Human Health. *Annual Review of Animal Biosciences*, *7*(1), 449–472. https://doi.org/10.1146/annurev-animal-030117-014523

Otto, S. P. (2018). Adaptation, speciation and extinction in the Anthropocene. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1891). https://doi.org/10.1098/rspb.2018.2047

Palmisano, A., Priami, C., & Tech, S. V. (2013). Encyclopedia of Systems Biology. *Encyclopedia of Systems Biology*. https://doi.org/10.1007/978-1-4419-9863-7

Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change. *Nature*, *421*, 37–42. https://doi.org/10.1038/nature01286

Rhodes, O. E., Bréchignac, F., Bradshaw, C., Hinton, T. G., Mothersill, C., Arnone, J. A., … Zimmerman, J. K. (2020). Integration of ecosystem science into radioecology : A consensus perspective. *Science of the Total Environment*, *740*, 140031. https://doi.org/10.1016/j.scitotenv.2020.140031

Singel, S. M., Cornelius, C., Batten, K., Fasciani, G., Wright, W. E., Lum, L., & Shay, J. W. (2013). A targeted RNAi screen of the breast cancer genome identifies KIF14 and TLN1 as genes that modulate docetaxel chemosensitivity in triple-negative breast cancer. *Clinical Cancer Research*, *19*(8), 2061–2070. https://doi.org/10.1158/1078-0432.CCR-13-0082

Slokar, G., & Hasler, G. (2016). Human endogenous retroviruses as pathogenic factors in the development of schizophrenia. *Frontiers in Psychiatry*, *6*(JAN), 1–10. https://doi.org/10.3389/fpsyt.2015.00183

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science (New York, N.Y.)*, *306*(October), 1783–1786. https://doi.org/10.1126/science.1103538

Subramanian, M. (2019). Humans versus Earth: the quest to define the Anthropocene. *Nature*, *572*(7768), 168–170. https://doi.org/10.1038/d41586-019-02381-2

Waterhouse, M. D., Erb, L. P., Beever, E. A., & Russello, M. A. (2018). Adaptive population divergence and directional gene flow across steep elevational gradients in a climate-sensitive mammal. *Molecular Ecology*, (March), 2512–2528. https://doi.org/10.1111/mec.14701

Whicker, F. W., & Schultz, V. (1982). Radioecology: Nuclear Energy and the Environment. *The Journal of Applied Ecology*, *21*(2), 733. https://doi.org/10.2307/2403460

Xia, L., Xiao, X., Liu, W. L., Song, Y., Liu, T. J. J., Li, Y. J., … Ben-David, Y. (2018). Coactosin-like protein CLP/Cotl1 suppresses breast cancer growth through activation of IL-24/PERP and inhibition of non-canonical TGFβ signaling. *Oncogene*, *37*(3), 323–331. https://doi.org/10.1038/onc.2017.342

Zhao, H., Ning, S., Nolley, R., Scicinski, J., Oronsky, B., Knox, S. J., & Peehl, D. M. (2017). The immunomodulatory anticancer agent, RRx-001, induces an interferon response through epigenetic induction of viral mimicry. *Clinical Epigenetics*, *9*(1), 1–11. https://doi.org/10.1186/s13148-017-0312-z

APPENDIX A

CHAPTER 2

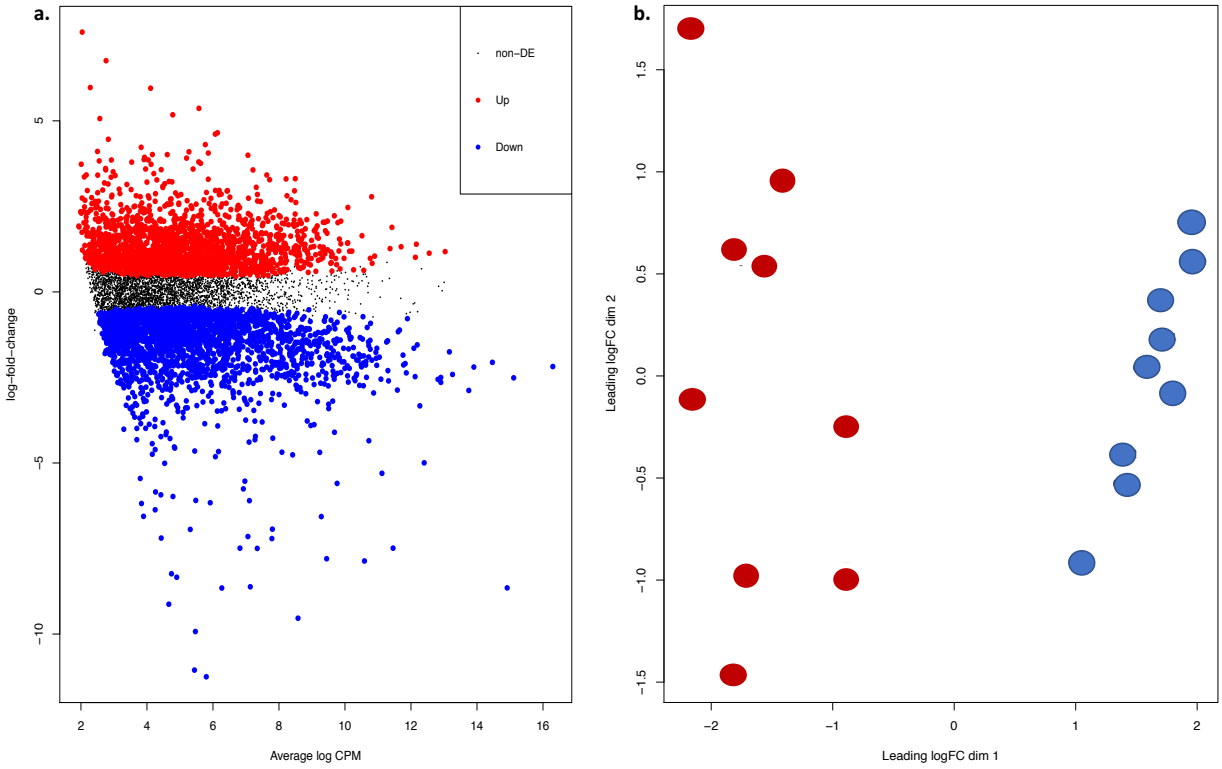

Figure A1. (**a.**) A smear plot of differentially expressed genes in CEZ wolves in comparison to wolves from northern Belarus, with up regulated genes in red and down regulated genes in blue. (**b**.)A multidimensional scaling (MDS) plot of gene expression depicts patterns of regulatory divergense between CEZ (blue) and northern Belarus (red).

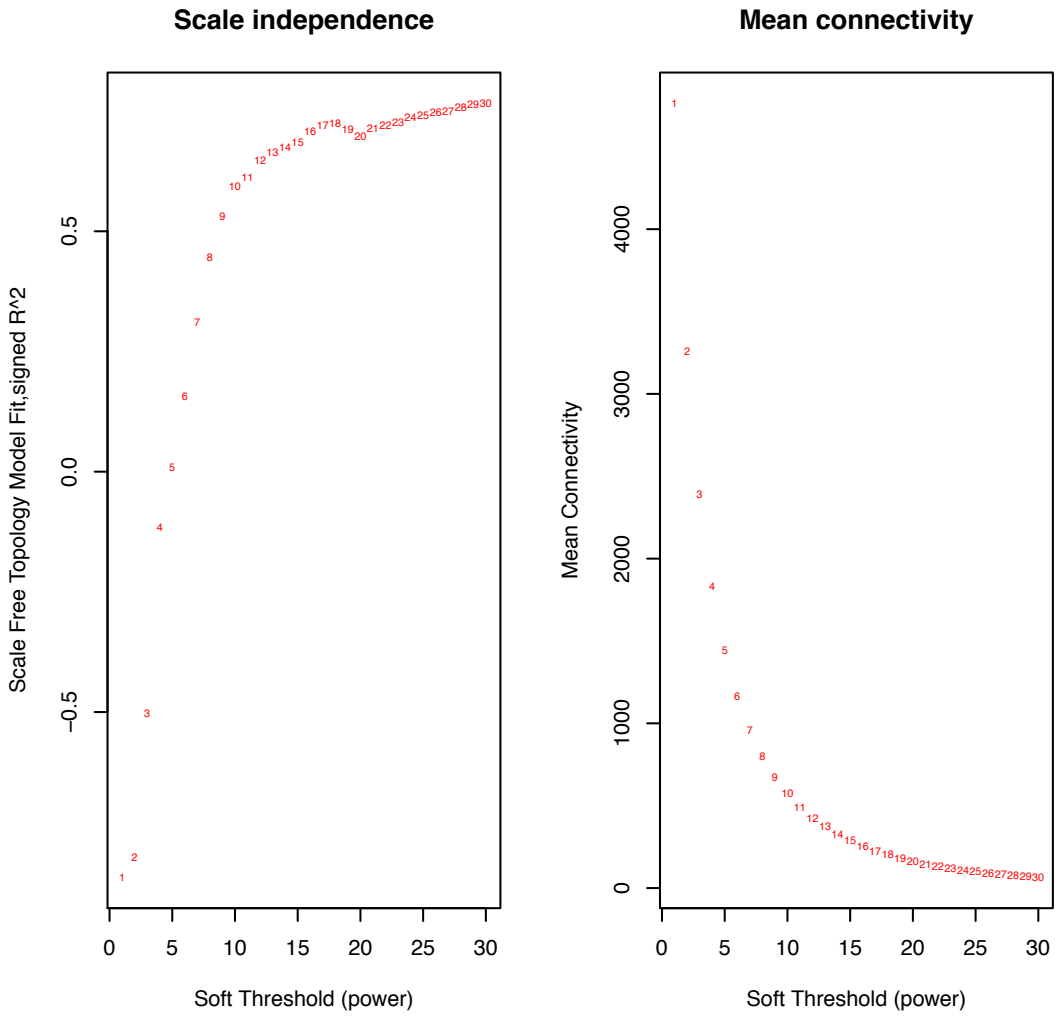

Figure A2. Network topology for select soft-thresholding powers. Described here are the scale-free fit topology index and mean connectivity as a function of the soft-thresholding power. We selected power 18 for our analyses, given the lack of scale-free topology fit and small sample numbers (Langfelder & Horvath, 2008).

Table A1. Functional enrichment of gene ontology (GO) terms from significant co-expression modules identified through weighted gene correlation network analyses (WGCNA) of gray wolf blood transcriptomes in the Chernobyl Exclusion Zone.

Module 1								
p value	***term size***	***query size***	***overlap size***	***precision***	***recall***	***term id***	***domain***	***term name***
0.00181	329	471	41	0.087	0.125	GO:0002376	BP	immune system process
0.0335	21	471	6	0.013	0.286	GO:0030098	BP	lymphocyte differentiation
0.0321	9	471	4	0.008	0.444	GO:0071025	BP	RNA surveillance
0.0316	605	471	59	0.125	0.098	GO:0006950	BP	response to stress
0.0462	30	471	7	0.015	0.233	GO:0022904	BP	respiratory electron transport chain
0.00115	103	471	19	0.04	0.184	GO:0007005	BP	mitochondrion organization
0.00583	6	471	4	0.008	0.667	GO:0001706	BP	endoderm formation
1.83E-17	267	471	64	0.136	0.24	GO:0022613	BP	ribonucleoprotein complex biogenesis

0.00387	78	471	15	0.032	0.192	GO:0009141	BP	nucleoside triphosphate metabolic process
0.0462	10	471	4	0.008	0.4	GO:0071428	BP	rRNA-containing ribonucleoprotein complex export from nucleus
0.0319	78	471	13	0.028	0.167	GO:0009123	BP	nucleoside monophosphate metabolic process
2.88E-21	4349	471	390	0.828	0.09	GO:0044424	CC	intracellular part
0.0422	206	471	25	0.053	0.121	GO:0140098	MF	catalytic activity, acting on RNA
2.63E-17	357	471	75	0.159	0.21	GO:0003723	MF	RNA binding
0.0422	5	471	3	0.006	0.6	GO:0043023	MF	ribosomal large subunit binding
5.25E-11	176	471	41	0.087	0.233	GO:0003735	MF	structural constituent of ribosome
0.0462	10	471	4	0.008	0.4	GO:0001054	MF	RNA polymerase I activity

0.00632	3	471	3	0.006	1	GO:0005471	MF	ATP:ADP antiporter activity
0.0435	21	200	6	0.03	0.286	HP:0004309	hp	Ventricular preexcitation
0.0163	11	200	5	0.025	0.455	HP:0001112	hp	Leber optic atrophy
0.00902	1766	200	127	0.635	0.072	HP:0025031	hp	Abnormality of the digestive system
0.0305	19	200	6	0.03	0.316	HP:0200043	hp	Verrucae
0.0435	239	200	26	0.13	0.109	HP:0004372	hp	Reduced consciousness/confusion
0.0414	63	200	11	0.055	0.175	HP:0000980	hp	Pallor
0.0297	100	200	15	0.075	0.15	HP:0002076	hp	Migraine
1.29E-07	424	200	57	0.285	0.134	HP:0010987	hp	Abnormality of cellular immune system
0.0435	74	200	12	0.06	0.162	HP:0002488	hp	Acute leukemia
0.0239	18	200	6	0.03	0.333	HP:0007763	hp	Retinal telangiectasia
0.00321	134	200	21	0.105	0.157	HP:0003128	hp	Lactic acidosis
0.0108	10	200	5	0.025	0.5	HP:0007768	hp	Central retinal vessel vascular tortuosity

0.0184	258	200	29	0.145	0.112	HP:0012378	hp	Fatigue
0.0376	72	200	12	0.06	0.167	HP:0011450	hp	CNS infection
0.0435	85	200	13	0.065	0.153	HP:0000245	hp	Abnormality of the paranasal sinuses
0.0435	46	200	9	0.045	0.196	HP:0001271	hp	Polyneuropathy
0.011	52	200	11	0.055	0.212	HP:0001427	hp	Mitochondrial inheritance
0.0127	22	200	7	0.035	0.318	HP:0003737	hp	Mitochondrial myopathy
0.0314	13	200	5	0.025	0.385	HP:0000576	hp	Centrocecal scotoma
0.0431	14	200	5	0.025	0.357	HP:0002401	hp	Stroke-like episode
0.0457	69	466	13	0.028	0.188	KEGG:04658	keg	Th1 and Th2 cell differentiation
0.0116	72	466	15	0.032	0.208	KEGG:04640	keg	Hematopoietic cell lineage
0.0424	25	466	7	0.015	0.28	KEGG:05330	keg	Allograft rejection
0.0339	123	466	20	0.043	0.163	KEGG:03013	keg	RNA transport
0.0116	31	466	9	0.019	0.29	KEGG:04940	keg	Type I diabetes mellitus
0.0111	29	466	9	0.019	0.31	KEGG:05340	keg	Primary immunodeficiency

0.000893	21	466	9	0.019	0.429	KEGG:05332	keg	Graft-versus-host disease
0.0116	135	466	23	0.049	0.17	KEGG:05010	keg	Alzheimer disease
0.000322	80	466	20	0.043	0.25	KEGG:04064	keg	NF-kappa B signaling pathway
0.00142	144	466	27	0.058	0.188	KEGG:05016	keg	Huntington disease
0.0497	70	466	13	0.028	0.186	KEGG:00240	keg	Pyrimidine metabolism
0.0116	43	466	11	0.024	0.256	KEGG:04612	keg	Antigen processing and presentation
0.000893	108	466	23	0.049	0.213	KEGG:00190	keg	Oxidative phosphorylation
0.0111	209	466	32	0.069	0.153	KEGG:05166	keg	Human T-cell leukemia virus 1 infection
0.0424	19	466	6	0.013	0.316	KEGG:03430	keg	Mismatch repair
5.85E-24	109	466	50	0.107	0.459	KEGG:03010	keg	Ribosome
0.0167	123	466	21	0.045	0.171	KEGG:04932	keg	Non-alcoholic fatty liver disease (NAFLD)
0.0188	40	466	10	0.021	0.25	KEGG:05134	keg	Legionellosis
2.39E-05	104	466	26	0.056	0.25	KEGG:03040	keg	Spliceosome

0.0291	72	466	14	0.03	0.194	KEGG:05323	keg	Rheumatoid arthritis
0.0116	185	466	29	0.062	0.157	KEGG:04714	keg	Thermogenesis
0.000109	114	466	26	0.056	0.228	KEGG:05012	keg	Parkinson disease
0.0111	29	466	9	0.019	0.31	KEGG:03030	keg	DNA replication
0.0099	24	522	7	0.013	0.292	REAC:R-CFA-180786	rea	Extension of Telomeres
4.29E-10	41	522	19	0.036	0.463	REAC:R-CFA-72702	rea	Ribosomal scanning and start codon recognition
0.0382	96	522	14	0.027	0.146	REAC:R-CFA-983705	rea	Signaling by the B Cell Receptor (BCR)
0.000684	134	522	23	0.044	0.172	REAC:R-CFA-69242	rea	S Phase
0.000285	76	522	17	0.033	0.224	REAC:R-CFA-198933	rea	Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell

0.0196	27	522	7	0.013	0.259	REAC:R-CFA-110314	rea	Recognition of DNA damage by PCNA-containing replication complex
0.00682	16	522	6	0.011	0.375	REAC:R-CFA-196757	rea	Metabolism of folate and pterines
0.00827	930	522	85	0.163	0.091	REAC:R-CFA-74160	rea	Gene expression (Transcription)
0.0322	94	522	14	0.027	0.149	REAC:R-CFA-15869	rea	Metabolism of nucleotides
8.33E-05	69	522	17	0.033	0.246	REAC:R-CFA-6781827	rea	Transcription-Coupled Nucleotide Excision Repair (TC-NER)
0.00835	30	522	8	0.015	0.267	REAC:R-CFA-5675221	rea	Negative regulation of MAPK pathway
1.39E-08	410	522	62	0.119	0.151	REAC:R-CFA-8953854	rea	Metabolism of RNA
0.00593	257	522	32	0.061	0.125	REAC:R-CFA-3700989	rea	Transcriptional Regulation by TP53

0.0257	91	522	14	0.027	0.154	REAC:R-CFA-202403	rea	TCR signaling
0.00461	14	522	6	0.011	0.429	REAC:R-CFA-110312	rea	Translesion synthesis by REV1
0.00279	169	522	25	0.048	0.148	REAC:R-CFA-1428517	rea	The citric acid (TCA) cycle and respiratory electron transport
0.00593	92	522	16	0.031	0.174	REAC:R-CFA-5668541	rea	TNFR2 non-canonical NF-kB pathway
Module 2								
p value	***term size***	***query size***	***overlap size***	***precision***	***recall***	***term id***	***domain***	***term name***
9.63E-05	4	198	4	0.02	1	GO:0072488	BP	ammonium transmembrane transport
0.0211	26	198	5	0.025	0.192	GO:0061726	BP	mitochondrion disassembly
0.0457	210	198	14	0.071	0.067	GO:0030163	BP	protein catabolic process
0.034	9	198	3	0.015	0.333	GO:0015695	BP	organic cation transport

0.0367	160	198	12	0.061	0.075	GO:0006511	BP	ubiquitin-dependent protein catabolic process
1.10E-06	7	198	6	0.03	0.857	GO:0006783	BP	heme biosynthetic process
1.94E-07	4471	198	166	0.838	0.037	GO:0005622	CC	intracellular
0.000259	5	198	4	0.02	0.8	GO:0016681	MF	oxidoreductase activity, acting on diphenols and related substances as donors, cytochrome as acceptor
0.00202	23	198	6	0.03	0.261	GO:0019843	MF	rRNA binding
0.0211	15	198	4	0.02	0.267	GO:0008519	MF	ammonium transmembrane transporter activity
0.0462	261	107	19	0.178	0.073	HP:0001332	hp	Dystonia
3.90E-07	8	107	7	0.065	0.875	HP:0010472	hp	Abnormality of the heme biosynthetic pathway

1.74E-06	84	107	16	0.15	0.19	HP:0011895	hp	Anemia due to reduced life span of red cells
0.0163	48	107	8	0.075	0.167	HP:0002904	hp	Hyperbilirubinemia
0.0305	482	107	29	0.271	0.06	HP:0001257	hp	Spasticity
0.00634	96	107	12	0.112	0.125	HP:0002134	hp	Abnormality of the basal ganglia
6.51E-05	27	196	8	0.041	0.296	KEGG:00860	keg	Porphyrin and chlorophyll metabolism
5.11E-09	108	196	21	0.107	0.194	KEGG:00190	keg	Oxidative phosphorylation
1.22E-07	185	196	25	0.128	0.135	KEGG:04714	keg	Thermogenesis
0.00129	1031	196	58	0.296	0.056	KEGG:01100	keg	Metabolic pathways
2.71E-05	109	196	16	0.082	0.147	KEGG:03010	keg	Ribosome
0.000132	39	196	9	0.046	0.231	KEGG:04216	keg	Ferroptosis
7.62E-12	144	196	28	0.143	0.194	KEGG:05016	keg	Huntington disease
3.94E-10	114	196	23	0.117	0.202	KEGG:05012	keg	Parkinson disease
0.0035	59	196	9	0.046	0.153	KEGG:04260	keg	Cardiac muscle contraction
3.91E-08	135	196	22	0.112	0.163	KEGG:05010	keg	Alzheimer disease

0.00413	119	196	13	0.066	0.109	KEGG:04723	keg	Retrograde endocannabinoid signaling
3.91E-08	123	196	21	0.107	0.171	KEGG:04932	keg	Non-alcoholic fatty liver disease (NAFLD)
0.0148	31	247	5	0.02	0.161	REAC:R-CFA-8866652	rea	Synthesis of active ubiquitin: roles of E1 and E2 enzymes
0.0311	4	247	2	0.008	0.5	REAC:R-CFA-8849469	rea	PTK6 Regulates RTKs and Their Effectors AKT1 and DOK1
0.0121	147	247	12	0.049	0.082	REAC:R-CFA-69481	rea	G2/M Checkpoints
6.11E-05	45	247	10	0.04	0.222	REAC:R-CFA-8939902	rea	Regulation of RUNX2 expression and activity
0.0145	94	247	9	0.036	0.096	REAC:R-CFA-9020702	rea	Interleukin-1 signaling

6.51E-10	120	247	23	0.093	0.192	REAC:R-CFA-163200	rea	Respiratory electron transport, ATP synthesis by chemiosmotic coupling, and heat production by uncoupling proteins.
0.00029	49	247	9	0.036	0.184	REAC:R-CFA-4641258	rea	Degradation of DVL
0.00029	48	247	9	0.036	0.188	REAC:R-CFA-8941858	rea	Regulation of RUNX3 expression and activity
0.000303	50	247	9	0.036	0.18	REAC:R-CFA-5610780	rea	Degradation of GLI1 by the proteasome
0.044	14	247	3	0.012	0.214	REAC:R-CFA-2173788	rea	Downregulation of TGF-beta receptor signaling
0.000791	1492	247	70	0.283	0.047	REAC:R-CFA-392499	rea	Metabolism of proteins
0.000392	53	247	9	0.036	0.17	REAC:R-CFA-5607761	rea	Dectin-1 mediated noncanonical NF-kB signaling

0.0443	156	247	11	0.045	0.071	REAC:R-CFA-2467813	rea	Separation of Sister Chromatids
0.000154	165	247	18	0.073	0.109	REAC:R-CFA-5689880	rea	Ub-specific processing proteases
0.000266	82	247	12	0.049	0.146	REAC:R-CFA-4086400	rea	PCP/CE pathway
0.00029	48	247	9	0.036	0.188	REAC:R-CFA-450408	rea	AUF1 (hnRNP D0) binds and destabilizes mRNA
0.0235	11	247	3	0.012	0.273	REAC:R-CFA-445095	rea	Interaction between L1 and Ankyrins
0.000125	61	247	11	0.045	0.18	REAC:R-CFA-68867	rea	Assembly of the pre-replicative complex
0.000526	69	247	10	0.04	0.145	REAC:R-CFA-5687128	rea	MAPK6/MAPK4 signaling
0.032	24	247	4	0.016	0.167	REAC:R-CFA-917729	rea	Endosomal Sorting Complex Required For Transport (ESCRT)

0.00029	62	247	10	0.04	0.161	REAC:R-CFA-8939236	rea	RUNX1 regulates transcription of genes involved in differentiation of HSCs
0.00029	48	247	9	0.036	0.188	REAC:R-CFA-8854050	rea	FBXL7 down-regulates AURKA during mitotic entry and in early mitosis
0.000791	74	247	10	0.04	0.135	REAC:R-CFA-2871837	rea	FCERI mediated NF-kB activation
0.00177	68	247	9	0.036	0.132	REAC:R-CFA-195253	rea	Degradation of beta-catenin by the destruction complex
0.000266	45	247	9	0.036	0.2	REAC:R-CFA-69601	rea	Ubiquitin Mediated Degradation of Phosphorylated Cdc25A
0.000117	60	247	11	0.045	0.183	REAC:R-CFA-8948751	rea	Regulation of PTEN stability and activity

0.046	5	247	2	0.008	0.4	REAC:R-CFA-2142712	rea	Synthesis of 12-eicosatetraenoic acid derivatives
0.00029	6	247	4	0.016	0.667	REAC:R-CFA-1247673	rea	Erythrocytes take up oxygen and release carbon dioxide
0.00923	55	247	7	0.028	0.127	REAC:R-CFA-5658442	rea	Regulation of RAS by GAPs
0.000392	53	247	9	0.036	0.17	REAC:R-CFA-5676590	rea	NIK-->noncanonical NF-kB signaling
0.000269	270	247	23	0.093	0.085	REAC:R-CFA-983168	rea	Antigen processing: Ubiquitination & Proteasome degradation
0.0148	19	247	4	0.016	0.211	REAC:R-CFA-5205685	rea	Pink/Parkin Mediated Mitophagy
0.000269	58	247	10	0.04	0.172	REAC:R-CFA-174084	rea	Autodegradation of Cdh1 by Cdh1:APC/C

Module 3								
p value	***term size***	***query size***	***overlap size***	***precision***	***recall***	***term id***	***domain***	***term name***
0.00101	4	18	2	0.111	0.5	GO:0035458	BP	cellular response to interferon-beta
7.55E-08	18	18	5	0.278	0.278	GO:0009615	BP	response to virus
0.000927	100	26	5	0.192	0.05	KEGG:05160	keg	Hepatitis C
0.0092	106	26	4	0.154	0.038	KEGG:05162	keg	Measles
7.20E-05	138	26	7	0.269	0.051	KEGG:05168	keg	Herpes simplex infection
0.00147	117	26	5	0.192	0.043	KEGG:04621	keg	NOD-like receptor signaling pathway
0.00025	121	26	6	0.231	0.05	KEGG:05164	keg	Influenza A
0.0299	16	32	2	0.062	0.125	REAC:R-CFA-197264	rea	Nicotinamide salvaging
0.0126	40	32	3	0.094	0.075	REAC:R-CFA-913531	rea	Interferon Signaling
2.17E-05	14	32	4	0.125	0.286	REAC:R-CFA-1236974	rea	ER-Phagosome pathway
0.0126	41	32	3	0.094	0.073	REAC:R-CFA-3108214	rea	SUMOylation of DNA damage response and repair proteins

Module 6								
p value	*term size*	*query size*	*overlap size*	*precision*	*recall*	*term id*	*domain*	*term name*
0.00527	262	1841	95	0.052	0.363	GO:0012501	BP	programmed cell death
0.034	9	1841	7	0.004	0.778	GO:0072666	BP	establishment of protein localization to vacuole
0.0433	16	1841	10	0.005	0.625	GO:0007062	BP	sister chromatid cohesion
0.0131	14	1841	10	0.005	0.714	GO:0031122	BP	cytoplasmic microtubule organization
0.034	7	1841	6	0.003	0.857	GO:0097286	BP	iron ion import
0.000334	22	1841	16	0.009	0.727	GO:0007034	BP	vacuolar transport
1.55E-07	216	1841	97	0.053	0.449	GO:0044093	BP	positive regulation of molecular function
0.0055	15	1841	11	0.006	0.733	GO:0051452	BP	intracellular pH reduction
2.58E-13	348	1841	159	0.086	0.457	GO:0016192	BP	vesicle-mediated transport

0.0106	91	1841	39	0.021	0.429	GO:0044087	BP	regulation of cellular component biogenesis
0.0126	122	1841	49	0.027	0.402	GO:0061025	BP	membrane fusion
0.0052	68	1841	32	0.017	0.471	GO:0098562	CC	cytoplasmic side of membrane
2.89E-05	93	1841	47	0.026	0.505	GO:0019898	CC	extrinsic component of membrane
5.73E-06	306	1841	122	0.066	0.399	GO:1990234	CC	transferase complex
5.53E-46	4471	1841	1428	0.776	0.319	GO:0005622	CC	intracellular
0.0263	15	1841	10	0.005	0.667	GO:0004559	MF	alpha-mannosidase activity
0.0224	17	1841	11	0.006	0.647	GO:0000030	MF	mannosyltransferase activity
1.60E-18	829	1841	334	0.181	0.403	GO:0140096	MF	catalytic activity, acting on a protein
0.00896	96	1841	41	0.022	0.427	GO:0003712	MF	transcription coregulator activity
0.0413	42	1841	20	0.011	0.476	GO:0008017	MF	microtubule binding

0.0273	5	1841	5	0.003	1	GO:0016922	MF	nuclear receptor binding
0.00275	135	1841	56	0.03	0.415	GO:0008047	MF	enzyme activator activity
4.27E-08	296	1841	126	0.068	0.426	GO:0019899	MF	enzyme binding
0.013	147	1008	62	0.062	0.422	HP:0001387	hp	Joint stiffness
0.0328	12	1008	9	0.009	0.75	HP:0010522	hp	Dyslexia
0.0254	917	1008	302	0.3	0.329	HP:0011138	hp	Abnormality of skin adnexa morphology
0.0291	8	1008	7	0.007	0.875	HP:0003409	hp	Distal sensory impairment of all modalities
0.0198	48	1008	25	0.025	0.521	HP:0001004	hp	Lymphedema
0.0156	114	1008	50	0.05	0.439	HP:0000154	hp	Wide mouth
0.0291	8	1008	7	0.007	0.875	HP:0010729	hp	Cherry red spot of the macula
0.0408	51	1008	25	0.025	0.49	HP:0000979	hp	Purpura
0.0175	17	1008	12	0.012	0.706	HP:0011012	hp	Abnormality of polysaccharide metabolism
0.00739	1143	1008	376	0.373	0.329	HP:0031797	hp	Clinical course

0.0175	193	1008	77	0.076	0.399	HP:0011830	hp	Abnormal oral mucosa morphology
0.0206	6	1008	6	0.006	1	HP:0001014	hp	Angiokeratoma
0.0291	8	1008	7	0.007	0.875	HP:0001212	hp	Prominent fingertip pads
0.0125	1081	1008	355	0.352	0.328	HP:0030680	hp	Abnormality of cardiovascular system morphology
0.0229	558	1008	193	0.191	0.346	HP:0002648	hp	Abnormality of calvarial morphology
0.0309	16	1008	11	0.011	0.688	HP:0009720	hp	Adenoma sebaceum
0.00127	204	1008	87	0.086	0.426	HP:0002079	hp	Hypoplasia of the corpus callosum
0.0465	5	1008	5	0.005	1	HP:0010746	hp	Hypoplasia of the phalanges of the toes
0.0282	882	1008	291	0.289	0.33	HP:0004322	hp	Short stature
0.0323	10	1008	8	0.008	0.8	HP:0002963	hp	Abnormal delayed hypersensitivity skin test
0.0449	73	1008	33	0.033	0.452	HP:0004400	hp	Abnormality of the pylorus

1.43E-07	1341	1008	471	0.467	0.351	HP:0003808	hp	Abnormal muscle tone
0.042	177	1008	69	0.068	0.39	HP:0003119	hp	Abnormality of lipid metabolism
0.0328	58	1008	28	0.028	0.483	HP:0000527	hp	Long eyelashes
0.0406	92	1008	40	0.04	0.435	HP:0002936	hp	Distal sensory impairment
0.0136	1360	1008	437	0.434	0.321	HP:0040064	hp	Abnormality of limbs
0.0465	5	1008	5	0.005	1	HP:0012269	hp	Abnormal muscle glycogen content
0.00404	8	1008	8	0.008	1	HP:0001922	hp	Vacuolated lymphocytes
0.00511	147	1008	64	0.063	0.435	HP:0000280	hp	Coarse facial features
0.0406	454	1008	158	0.157	0.348	HP:0009830	hp	Peripheral neuropathy
0.02	43	1008	23	0.023	0.535	HP:0002208	hp	Coarse hair
0.025	1975	1008	611	0.606	0.309	HP:0000478	hp	Abnormality of the eye
0.0426	98	1008	42	0.042	0.429	HP:0010786	hp	Urinary tract neoplasm
0.00139	52	1008	30	0.03	0.577	HP:0000307	hp	Pointed chin
0.025	143	1008	59	0.059	0.413	HP:0001007	hp	Hirsutism
0.00954	14	1008	11	0.011	0.786	HP:0006562	hp	Viral hepatitis

0.00241	351	1008	135	0.134	0.385	HP:0000288	hp	Abnormality of the philtrum
0.00994	58	1008	30	0.03	0.517	HP:0001640	hp	Cardiomegaly
0.0156	47	1008	25	0.025	0.532	HP:0005584	hp	Renal cell carcinoma
0.0127	72	1008	35	0.035	0.486	HP:0000574	hp	Thick eyebrow
0.0328	66	1008	31	0.031	0.47	HP:0200034	hp	Papule
0.00223	89	1662	42	0.025	0.472	KEGG:04659	keg	Th17 cell differentiation
0.0258	107	1662	44	0.026	0.411	KEGG:04926	keg	Relaxin signaling pathway
0.00331	42	1662	23	0.014	0.548	KEGG:00510	keg	N-Glycan biosynthesis
0.0108	260	1662	98	0.059	0.377	KEGG:05165	keg	Human papillomavirus infection
0.00285	77	1662	37	0.022	0.481	KEGG:05215	keg	Prostate cancer
0.00198	60	1662	31	0.019	0.517	KEGG:01524	keg	Platinum drug resistance
0.00552	121	1662	52	0.031	0.43	KEGG:05164	keg	Influenza A
0.0396	64	1662	28	0.017	0.438	KEGG:03018	keg	RNA degradation
0.0129	46	1662	23	0.014	0.5	KEGG:04370	keg	VEGF signaling pathway
0.00384	100	1662	45	0.027	0.45	KEGG:05160	keg	Hepatitis C

0.00198	65	1662	33	0.02	0.508	KEGG:05100	keg	Bacterial invasion of epithelial cells
1.17E-12	108	1662	71	0.043	0.657	KEGG:04142	keg	Lysosome
0.0093	93	1662	41	0.025	0.441	KEGG:04919	keg	Thyroid hormone signaling pathway
0.00223	116	1662	52	0.031	0.448	KEGG:04145	keg	Phagosome
0.018	128	1662	52	0.031	0.406	KEGG:04218	keg	Cellular senescence
0.0227	67	1662	30	0.018	0.448	KEGG:01521	keg	EGFR tyrosine kinase inhibitor resistance
0.00198	55	1662	29	0.017	0.527	KEGG:05223	keg	Non-small cell lung cancer
0.0314	31	1662	16	0.01	0.516	KEGG:00051	keg	Fructose and mannose metabolism
0.000348	151	1662	68	0.041	0.45	KEGG:04062	keg	Chemokine signaling pathway
0.0211	242	1662	90	0.054	0.372	KEGG:04010	keg	MAPK signaling pathway

1.39E-05	143	1662	70	0.042	0.49	KEGG:05167	keg	Kaposi sarcoma-associated herpesvirus infection
6.13E-10	198	1662	104	0.063	0.525	KEGG:04144	keg	Endocytosis
4.75E-05	109	1662	55	0.033	0.505	KEGG:04217	keg	Necroptosis
0.00854	426	1662	153	0.092	0.359	KEGG:05200	keg	Pathways in cancer
0.00315	30	1662	18	0.011	0.6	KEGG:04136	keg	Autophagy - other
0.000135	17	1662	14	0.008	0.824	KEGG:00514	keg	Other types of O-glycan biosynthesis
0.0067	62	1662	30	0.018	0.484	KEGG:05132	keg	Salmonella infection
0.00384	55	1662	28	0.017	0.509	KEGG:04520	keg	Adherens junction
0.00132	116	1662	53	0.032	0.457	KEGG:04910	keg	Insulin signaling pathway
1.39E-05	100	1662	53	0.032	0.53	KEGG:04380	keg	Osteoclast differentiation
0.00132	28	1662	18	0.011	0.643	KEGG:04215	keg	Apoptosis - multiple species
0.00256	56	1662	29	0.017	0.518	KEGG:04137	keg	Mitophagy - animal
0.000209	101	1662	50	0.03	0.495	KEGG:04611	keg	Platelet activation

0.00252	87	1662	41	0.025	0.471	KEGG:04660	keg	T cell receptor signaling pathway
1.39E-06	117	1662	63	0.038	0.538	KEGG:04621	keg	NOD-like receptor signaling pathway
2.84E-06	117	1662	62	0.037	0.53	KEGG:04140	keg	Autophagy - animal
0.0314	114	1662	46	0.028	0.404	KEGG:04728	keg	Dopaminergic synapse
0.0159	130	1662	53	0.032	0.408	KEGG:04150	keg	mTOR signaling pathway
4.45E-05	101	1662	52	0.031	0.515	KEGG:04071	keg	Sphingolipid signaling pathway
0.00285	112	1662	50	0.03	0.446	KEGG:04152	keg	AMPK signaling pathway
0.000519	164	1662	72	0.043	0.439	KEGG:04810	keg	Regulation of actin cytoskeleton
5.20E-05	170	1662	78	0.047	0.459	KEGG:05163	keg	Human cytomegalovirus infection
0.0404	104	1662	42	0.025	0.404	KEGG:04915	keg	Estrogen signaling pathway
0.0153	15	1662	10	0.006	0.667	KEGG:00511	keg	Other glycan degradation

1.35E-05	102	1662	54	0.032	0.529	KEGG:04722	keg	Neurotrophin signaling pathway
0.000438	63	1662	34	0.02	0.54	KEGG:05220	keg	Chronic myeloid leukemia
0.00383	89	1662	41	0.025	0.461	KEGG:04625	keg	C-type lectin receptor signaling pathway
0.000286	110	1662	53	0.032	0.482	KEGG:04068	keg	FoxO signaling pathway
0.0265	148	1662	58	0.035	0.392	KEGG:05169	keg	Epstein-Barr virus infection
2.84E-06	117	1662	62	0.037	0.53	KEGG:04210	keg	Apoptosis
0.0159	60	1662	28	0.017	0.467	KEGG:04917	keg	Prolactin signaling pathway
8.07E-10	141	1662	80	0.048	0.567	KEGG:04141	keg	Protein processing in endoplasmic reticulum
0.00441	71	1662	34	0.02	0.479	KEGG:04012	keg	ErbB signaling pathway
0.00123	76	1662	38	0.023	0.5	KEGG:04620	keg	Toll-like receptor signaling pathway

8.66E-05	71	1662	39	0.023	0.549	KEGG:05210	keg	Colorectal cancer
1.07E-05	84	1662	47	0.028	0.56	KEGG:04070	keg	Phosphatidylinositol signaling system
0.016	71	1662	32	0.019	0.451	KEGG:04912	keg	GnRH signaling pathway
0.00118	118	1662	54	0.032	0.458	KEGG:04120	keg	Ubiquitin mediated proteolysis
0.047	51	1662	23	0.014	0.451	KEGG:04213	keg	Longevity regulating pathway - multiple species
0.00556	64	1662	31	0.019	0.484	KEGG:05212	keg	Pancreatic cancer
0.0449	40	1662	19	0.011	0.475	KEGG:05134	keg	Legionellosis
0.0159	52	1662	25	0.015	0.481	KEGG:05230	keg	Central carbon metabolism in cancer
0.00252	95	1662	44	0.026	0.463	KEGG:05145	keg	Toxoplasmosis
4.45E-05	161	1662	75	0.045	0.466	KEGG:05170	keg	Human immunodeficiency virus 1 infection

0.00228	76	1662	37	0.022	0.487	KEGG:04211	keg	Longevity regulating pathway
0.0109	80	1662	36	0.022	0.45	KEGG:04922	keg	Glucagon signaling pathway
6.31E-06	137	1662	69	0.042	0.504	KEGG:05152	keg	Tuberculosis
0.000519	49	1662	28	0.017	0.571	KEGG:05213	keg	Endometrial cancer
0.0159	130	1662	53	0.032	0.408	KEGG:05225	keg	Hepatocellular carcinoma
0.0378	92	1662	38	0.023	0.413	KEGG:05142	keg	Chagas disease (American trypanosomiasis)
0.000286	57	1662	32	0.019	0.561	KEGG:05221	keg	Acute myeloid leukemia
0.00777	106	1662	46	0.028	0.434	KEGG:05162	keg	Measles
0.00375	147	1662	62	0.037	0.422	KEGG:05203	keg	Viral carcinogenesis
0.0159	165	1662	65	0.039	0.394	KEGG:04510	keg	Focal adhesion
3.42E-07	63	1662	41	0.025	0.651	KEGG:00562	keg	Inositol phosphate metabolism
0.016	82	1662	36	0.022	0.439	KEGG:05231	keg	Choline metabolism in cancer

0.0488	114	1662	45	0.027	0.395	KEGG:05418	keg	Fluid shear stress and atherosclerosis
0.00288	59	1662	30	0.018	0.508	KEGG:05211	keg	Renal cell carcinoma
0.00139	87	1662	42	0.025	0.483	KEGG:04668	keg	TNF signaling pathway
2.20E-05	109	1662	56	0.034	0.514	KEGG:05161	keg	Hepatitis B
1.23E-05	68	1662	40	0.024	0.588	KEGG:04666	keg	Fc gamma R-mediated phagocytosis
8.63E-05	52	1662	31	0.019	0.596	KEGG:04664	keg	Fc epsilon RI signaling pathway
0.00139	69	1662	35	0.021	0.507	KEGG:04650	keg	Natural killer cell mediated cytotoxicity
2.03E-05	58	1662	35	0.021	0.603	KEGG:04662	keg	B cell receptor signaling pathway
0.0378	29	1662	15	0.009	0.517	KEGG:00052	keg	Galactose metabolism
0.00556	46	1662	24	0.014	0.522	KEGG:04622	keg	RIG-I-like receptor signaling pathway
0.0299	17	2388	11	0.005	0.647	REAC:R-CFA-5621575	rea	CD209 (DC-SIGN) signaling

2.66E-05	283	2388	122	0.051	0.431	REAC:R-CFA-8953897	rea	Cellular responses to external stimuli
0.00278	51	2388	28	0.012	0.549	REAC:R-CFA-373755	rea	Semaphorin interactions
0.0383	11	2388	8	0.003	0.727	REAC:R-CFA-162658	rea	Golgi Cisternae Pericentriolar Stack Reorganization
0.0299	17	2388	11	0.005	0.647	REAC:R-CFA-6804115	rea	TP53 regulates transcription of additional cell cycle genes whose exact role in the p53 pathway remain uncertain
0.0181	10	2388	8	0.003	0.8	REAC:R-CFA-429947	rea	Deadenylation of mRNA
1.92E-06	357	2388	153	0.064	0.429	REAC:R-CFA-194315	rea	Signaling by Rho GTPases
0.0383	11	2388	8	0.003	0.727	REAC:R-CFA-264876	rea	Insulin processing
0.0384	9	2388	7	0.003	0.778	REAC:R-CFA-112411	rea	MAPK1 (ERK2) activation

0.00136	33	2388	21	0.009	0.636	REAC:R-CFA-114604	rea	GPVI-mediated activation cascade
0.0333	15	2388	10	0.004	0.667	REAC:R-CFA-70221	rea	Glycogen breakdown (glycogenolysis)
0.0366	13	2388	9	0.004	0.692	REAC:R-CFA-8849471	rea	PTK6 Regulates RHO GTPases, RAS GTPase and MAP kinases
0.00275	10	2388	9	0.004	0.9	REAC:R-CFA-8875555	rea	MET activates RAP1 and RAC1
0.0383	11	2388	8	0.003	0.727	REAC:R-CFA-200425	rea	Import of palmitoyl-CoA into the mitochondrial matrix
0.0181	12	2388	9	0.004	0.75	REAC:R-CFA-196299	rea	Beta-catenin phosphorylation cascade
9.87E-38	1442	2388	641	0.268	0.445	REAC:R-CFA-168256	rea	Immune System
0.0462	45	2388	22	0.009	0.489	REAC:R-CFA-5628897	rea	TP53 Regulates Metabolic Genes

0.00975	28	2388	17	0.007	0.607	REAC:R-CFA-6804758	rea	Regulation of TP53 Activity through Acetylation
1.63E-06	88	2388	51	0.021	0.58	REAC:R-CFA-4420097	rea	VEGFA-VEGFR2 Pathway
1.53E-07	70	2388	45	0.019	0.643	REAC:R-CFA-1483255	rea	PI Metabolism
0.0277	26	2388	15	0.006	0.577	REAC:R-CFA-2871809	rea	FCERI mediated Ca+2 mobilization
0.00375	125	2388	56	0.023	0.448	REAC:R-CFA-73887	rea	Death Receptor Signalling
0.00687	56	2388	29	0.012	0.518	REAC:R-CFA-70171	rea	Glycolysis
5.52E-21	528	2388	261	0.109	0.494	REAC:R-CFA-199991	rea	Membrane Trafficking
0.00275	10	2388	9	0.004	0.9	REAC:R-CFA-2470946	rea	Cohesin Loading onto Chromatin
0.0253	33	2388	18	0.008	0.545	REAC:R-CFA-2871796	rea	FCERI mediated MAPK activation
0.00901	11	2388	9	0.004	0.818	REAC:R-CFA-3371568	rea	Attenuation phase
3.53E-09	177	2388	94	0.039	0.531	REAC:R-CFA-3247509	rea	Chromatin modifying enzymes

0.0181	81	2388	37	0.015	0.457	REAC:R-CFA-5357801	rea	Programmed Cell Death
0.000412	51	2388	30	0.013	0.588	REAC:R-CFA-397795	rea	G-protein beta:gamma signalling
0.0216	35	2388	19	0.008	0.543	REAC:R-CFA-8939243	rea	RUNX1 interacts with co-factors whose precise effect on RUNX1 targets is not known

Table A2: Correlations of gene pathway(Kanehisa, 2019) regulatory patterns and internal radiocesium activity in wolves from the CEZ.

Pathway	Correlation with IRA (p-value)	% DE genes
Base pair mismatch repair	0.000118	63.16%
Cytokine-cytokine receptor	7.84E-05	70.12%
Chemical carcinogenesis	0.000294	57.14%
Chronic myeloid leukemia Pathway	0.000118	53.33%
Acute myeloid leukemia	4.39E-05	58%
Transcriptional missregulation in cancer	7.37E-05	53.12%

APPENDIX B

CHAPTER 3

Table B1. Microparasites tested form using qPCR in wolves and raccoon dogs from the Chernobyl Exclusion Zone.

Pathogen	Gray Wolf		Raccoon Dog	
	N. Belarus	CEZ	N. Belarus	CEZ
Positive				
Anaplasma phagoctophilum	0	0.1	0	0
Babesia canis canids and *Babesia conradae*	0	0.1	0	0.85
Canine distemper virus	0.25	0	0	0
Coronavirus	0.125	0	0	0
Hepatozoon canis	0.5	0.2	0	0
Rickettsia spp.	0	0	0	0.33
Streptococcus equi zooepidemicus	0	0	0	0.33
Negative				
Blastomyces dermatitidis	0	0	0	0
Bordetella bronchiseptica	0	0	0	0
Brucella canis	0	0	0	0
Canine adenovirus type 2	0	0	0	0
Canine Bartonella	0	0	0	0
Canine herpes virus	0	0	0	0
Canine parainfluenza virus	0	0	0	0
Canine pneumovirus	0	0	0	0
Coccidioides spp.	0	0	0	0
Cryptococcus spp.	0	0	0	0
Ehrlichia spp.	0	0	0	0
Histoplasma capsulatum	0	0	0	0
Influenza A (H3N2) virus	0	0	0	0
Influenza A PCR	0	0	0	0
Leishmania spp.	0	0	0	0
Leptospira sp.	0	0	0	0
Leptospira sp.	0	0	0	0
Mycoplasma cynos	0	0	0	0
Neospora caninum	0	0	0	0
Toxoplasma gondii	0	0	0	0
Trypanosoma cruzi	0	0	0	0

APPENDIX C

CHAPTER 5

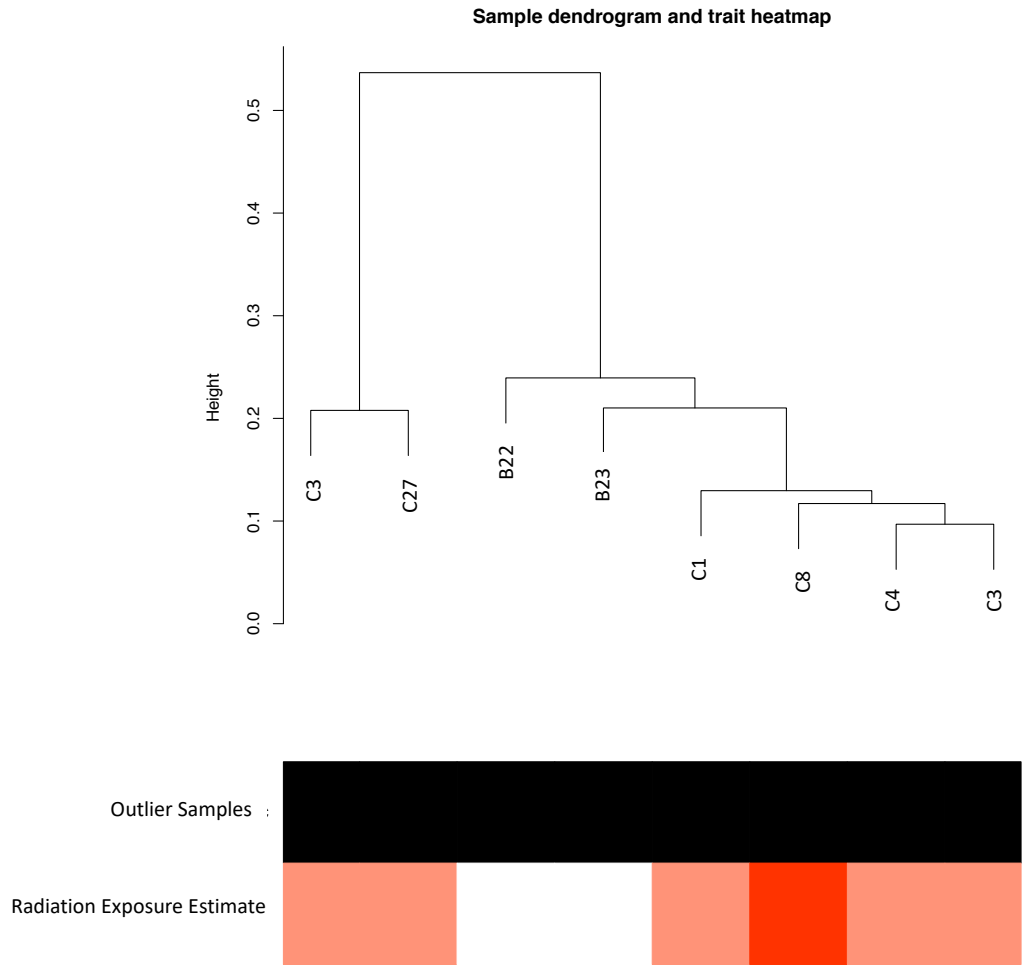

Figure C1: Hierarchical cluster plot of global transcription in raccoon dogs. No samples appear as outliers. Radiation exposure estimate heatmap is in the red gradient with samples from norther Belarus (B22 and B23) having the lowest estimated exposure.

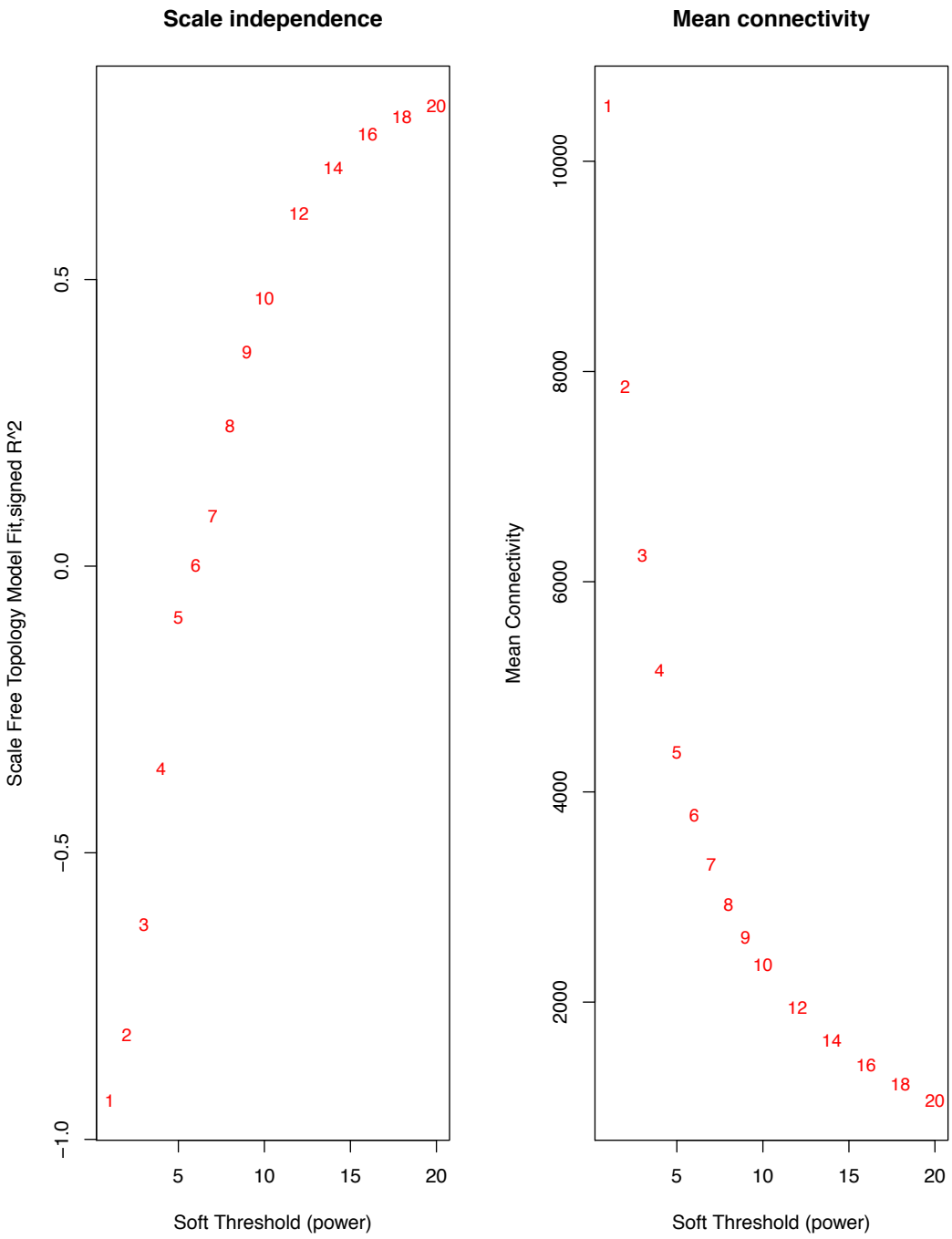

Figure C2: Network topology of raccoon dog transcriptome for identifying most appropriate soft-thresholding powers. On the left is the scale-free fit topology across soft-thresholding power, and on the right is mean connectivity as a function of soft-thresholding power.

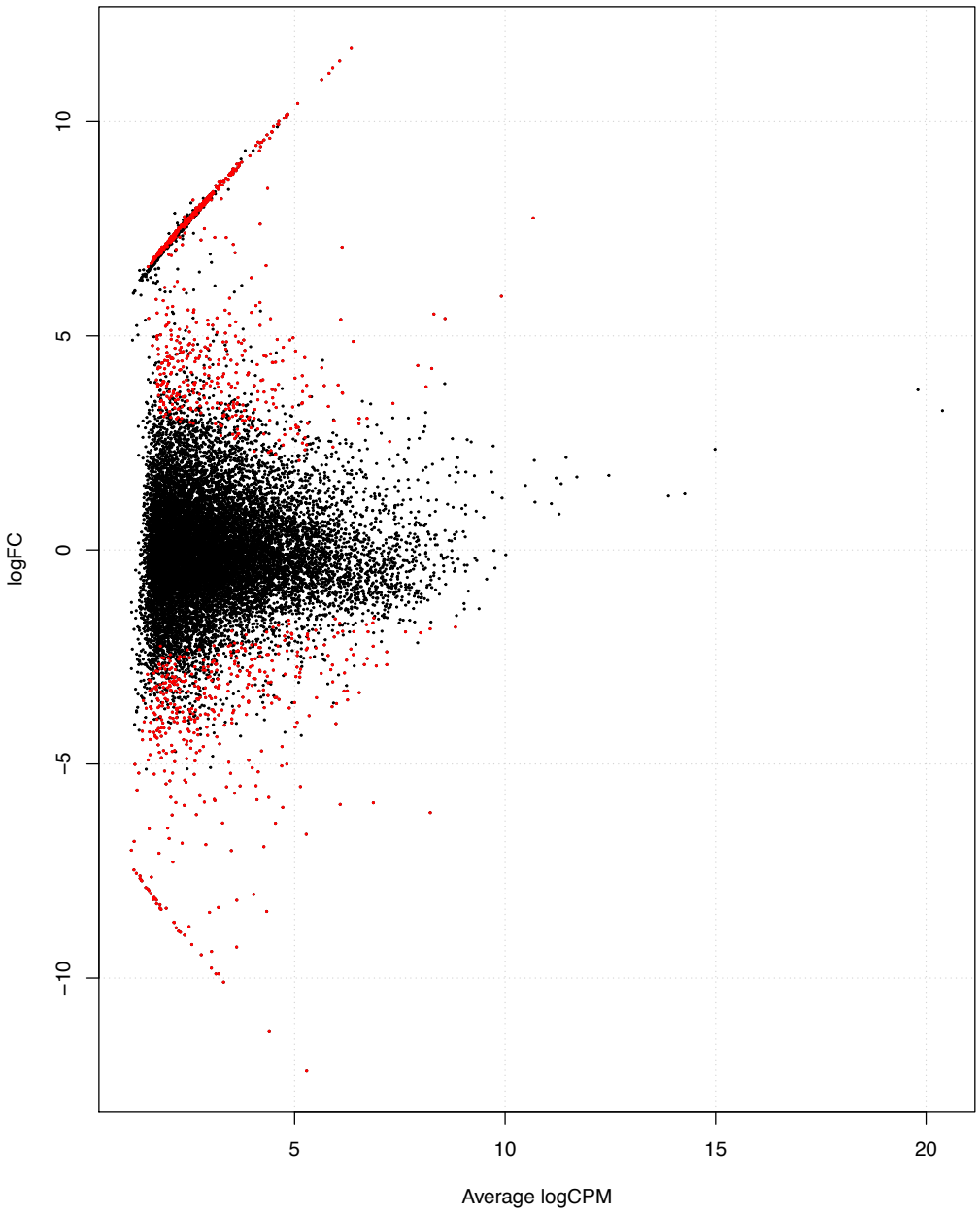

Figure C3. Smear plot of differentially expressed genes (in red) in the blood transcriptome of raccoon dogs from the CEZ