Fiscal Year:	FY 2020	**Task Last Updated:**	FY 05/15/2020
PI Name:	Fox, Donald Ph.D.		
Project Title:	Mining Biology's Extremes for New Space Radiation Resistance Strategies		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	TRISH--TRISH		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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City:	Durham	**State:**	NC
Zip Code:	27710	**Congressional District:**	1
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2017 HERO NNJ16ZSA001N-TRIRT. Appendix C: Translational Research Institute for Space Health (TRISH) Research Topics
Start Date:	10/01/2017	**End Date:**	12/31/2020
No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	1	**No. of Master' Degrees:**	0
No. of Master's Candidates:	1	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	2	**Monitoring Center:**	TRISH
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:	NOTE: End date changed to 12/31/2020 per TRISH (Ed., 6/17/2020)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Kirsch, David M.D., Ph.D. (Duke University Medical Center)		
Grant/Contract No.:	NNX16AO69A-T0108		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	The purpose of this solicitation is to uncover new understanding of how a species withstands space-relevant radiation exposure, using validation and safety efficacy studies in model organisms. Drosophila is specifically mentioned, and we have expertise in study of Drosophila radiation resistance mechanisms (Bretscher and Fox 2016, Dev Cell). As outlined in the solicitation, we will perform genetic manipulation in vivo in flies, targeting potential Tardigrade resilience mechanisms. Finally, the solicitation discusses follow-up work in rodents, which we are well-equipped to do, as Duke co-investigator Dr. Kirsch has prior NASA-funded experience in studying space radiation effects in mice at Brookhaven NASA Space Radiation Laboratory (NSRL). Reference: Bretscher, H. S. & Fox, D. T. Proliferation of double-strand break-resistant polyploid cells requires Drosophila FANCD2. Dev Cell 37, 444–457 (2016).
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	After year 2 of our proposal we have established 55 Fruit Fly lines expressing individual Tardigrade genes. These transgenic flies represent a new resource for the study of Tardigrade gene products and their potential impact on the biology of extreme environmental stress resistance, including resistance to radiation.
Task Progress:	[Ed. note May 2020: Report submitted by TRISH to Task Book in March 2020; covers reporting as of August 2019.] Results AIM1-- As outlined in our proposal, we aim to identify single Tardigrade genes that, when expressed in another organism, confer increased radiation resistance. We proposed to use Drosophila to rapidly screen through single Tardigrade genes in a whole organism context. In year two, we screened 47 independent transgenic fly lines for increased resistance to X-ray or 56Fe radiation. Each transgenic line expressed a single Tardigrade gene. Based on lifespan analysis over a 20 day period, ~15% of these lines exhibit at least a 25% reduction in baseline survival relative to isogenic controls, 75% of lines showed a 1-24% reduction in baseline survival, while 10% lines showed a potentially mild improvement in survival. We subjected each line to a survival analysis following a single dose of radiation, and we also included isogenic controls in each radiation experiment. A minimum of 50 animals were scored/radiation trial. Based on dose response studies performed in years 1 and 2 (X-ray studies at Duke and 56Fe studies at NASA Space Radiation Laboratory (NSRL)), we established 15 Gy for both radiation sources as a dose that reproducibly leads to about 50% lethality in 20 day old adults when animals are irradiated during the third larval instar. Of the lines examined so far, 4 show promising effects in terms of increased radiation resistance for at least one radiation source. Each of these lines resulted in approximately a 20% increase in survival relative to isogenic control lines, and for X-ray these candidate radioprotectors showed similar improvement in animal survival in two replicate trials. All 4 lines express genes related to superoxide dismutase (SOD) biology. The same SOD genes identified so far as radioprotective are not the same between X-ray and 56Fe, which could preliminarily suggest differences between protective responses to terrestrial and galactic cosmic radiation. While survival of candidate radioprotective line D2-2 suggests this line may cause a decrease in organismal health in the absence of radiation, the other three candidate radioprotective lines show no significant decrease in fitness, with line F3-2 showing a potentially mild increase in fitness. In the coming year we see no issues with meeting our proposal's stated goal of establishing ~100 transgenic lines, each expressing a Tardigrade-unique gene, and testing each line's ability to confer increased organismal health following X-ray and 56Fe radiation. We anticipate having at least one analysis of each transgenic line for 56Fe following our November 13th, 2019 irradiation at NSRL, and plan an additional NSRL radiation for further replicate analyses in Spring of 2020. At that time, we also plan to subject several lines to 16O radiation, to examine the effect of expressing Tardigrade genes on a second source of galactic cosmic radiation. In the coming year, we are also interested in performing further analyses to determine whether these Tardigrade genes are better than Drosophila SOD genes at conferring radiation resistance, or whether they might synergize with expression of Drosophila SOD genes to improve radiation resistance. Additionally, our collaborators in the Kirsch lab will express our four top candidates in mammalian cells to determine if they observe a similar heightened radiation resistance. AIM2- As outlined in our proposal, we plan to conduct an unbiased screen for genes that are required for DNA damage resistance in the Drosophila hindgut. In years 1 and 2, we established 1300 independent lines carrying recessive lethal mutations on the X-chromosome. We are maintaining these lines as heterozygotes, but the lines are in a background that permits site-specific recombination on the X-chromosome to yield homozygous mutant hindgut cells. In year two, we encountered issues generating homozygous mutant hindgut cells while also expressing the I-Cre enzyme, which is the source of DNA damage in this screen. This is likely due to the use of repeated heat shocks in our screen. We perform one heat shock at the first larval instar stage to induce homozygous mutant tissue and a second heat shock at the second larval instar stage to activate the I-Cre enzyme at the desired timeframe, which is based on our previously published work (Bretscher and Fox, 2016). To resolve this technical hurdle, in year 2 we have developed a new fly line that lets us induce homozygous mutant cells using a non heat-shock method (the Gal4/UAS system). Based on our preliminary tests with this line, we now appear to have generated all of the fly lines needed to conduct the Aim2 screen within the next 6-8 months. Reference: Bretscher, H. S. & Fox, D. T. Proliferation of double-strand break-resistant polyploid cells requires Drosophila FANCD2. Dev Cell 37, 444–457 (2016).
Bibliography Type:	Description: (Last Updated: 09/04/2023)