Revealing the Role of Genetics in the Adaptation of Mammals to Spaceflight Factors

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# Purpose

# The purpose of this paper is to highlight the lack of information about the genetic influences on adaptation to the spaceflight environment, and to propose a framework for addressing this knowledge gap through a combination of ground studies and targeted spaceflight studies with rodents. We outline an example study that would identify the genetic determinants of bone remodeling and suggest that a similar approach could be used to tackle additional knowledge gaps.

This paper is a companion to two additional white papers on the application of quantitative genetics approaches to key space biology questions:

Advancing Space Life Science Research Using *Drosophila melanogaster*. Trudy Mackay, Janani Iyer, Siddhita D. Mhatre

Plant Quantitative Genomics in the Spaceflight Environment. Wolfgang Busch, Anna-Lisa Paul, Marcio Resende.

# Background

The spaceflight environment, including microgravity, radiation, confinement, and other stressors, presents a number of health challenges. Mammalian model organisms can be studied to address health-relevant questions regarding adaptation to the spaceflight environment, and to develop countermeasures.  However, two limitations to the use of rodents in adaptation/countermeasure studies exist: 1) Not all relevant aspects of the spaceflight environment can be faithfully replicated on Earth (e.g., microgravity or its consequences) and 2) Limitations in the numbers of animals that can be flown have resulted in most experiments being performed with animals from a single inbred strain, leading to potentially misleading conclusions (Sittig 2016) and precluding a deeper understanding of the role of genetics in spaceflight adaptation. Our broad proposal is that ground-based studies could be carried out to generate hypotheses regarding mammalian genetic and genomic influences on spaceflight adaptation, and that targeted (smaller sample size) spaceflight studies could be done to test these hypotheses.

## Strengths of Rodent Models

The choice of species for these studies will depend on the phenotype under study. While invertebrates or simple vertebrates may be useful for certain experimental questions, we anticipate that many biomedically important traits will require mammalian organisms to assure the greatest likelihood of translation to humans. The two most likely choices will be mice and rats, both of which have extremely well-developed genetic resources, including well-annotated genomes and transcriptomes as well as the availability of inbred and well-characterized outbred populations. In general, mice are typically used when possible because of their smaller size, which would be an especially important consideration when sending animals to space. However, rats are preferable for a number of behavioral and physiological traits where their greater behavioral repertoire and larger size are important advantages (Solberg Woods 2019). In particular, outbred populations like diversity outbred (DO) and HS/Npt heterogeneous stock mice and the N/NIH heterogeneous stock (HS) rats offer outstanding populations for mapping genetically complex traits (Saul 2019, find or delete this reference). Several studies have successfully mapped complex traits and identified underlying causal genes using both DO mice (Recla 2019, Ouellette 2020, Recla 2014) and HS rats (Rat Genome Sequencing and Mapping Consortium 2014, Tsaih 2014, Keele 2018, Chitre 2020, Keele 2021), demonstrating utility of these models. Similar successes have also been shown in commercial outbred mice (Parker 2016, Nicod 2016) as well as advanced intercross lines (Gonzales 2018). In addition, laboratory mice and rats also offer large panels of inbred strains, which can be useful when the interaction between an environmental manipulation and genotypes are under study.

## Previous uses of Quantitative Genetics for Space Biology

We are aware of one example in which NASA has used the tools of quantitative genetics to answer fundamental questions about a health risk associated with spaceflight (Edmondson 2020). High-charge, high-energy (HZE) ions are a component of space radiation. Their health effects on humans are unknown because life on Earth is shielded from HZE ions by the Earth’s magnetic field.  Even astronauts aboard the ISS are afforded considerable protection.  However, beyond low Earth orbit that protection is lost and since HZE ions are capable of penetrating any practical level of spacecraft shielding.  Therefore, astronauts on lunar or interplanetary missions will be exposed to significant levels of HZE ions.  To date, NASA's only human experience with HZE ions at fluences found in deep space comes from astronauts who flew Apollo lunar missions.  But those missions were of very short duration, so the exposure doses were only a small fraction of what will be experienced by astronauts on currently envisioned missions.

The NASA Space Radiation Laboratory is a ground-based facility that provides investigators the capability to simulate space radiation, including HZE ions.  Exploiting this capability, Edmondson and colleagues (Edmondson 2020) performed a study to explore the relationship between genotype and susceptibility to radiation-induced cancers. They used a cohort of more than 1,800 outbred heterogeneous stock mice, divided into three treatment groups.  One group was irradiated with gamma rays, a common form of radiation whose health effects are relatively well understood.  Another group was irradiated with HZE ions and the third group was a sham control that was not irradiated.  The mice were monitored for tumors until they reached 800 days of age and any tumors that arose were characterized by histopathology. The histopathologic results indicate that HZE ions do not cause unique tumors. Using SNP genotype data, 51 quantitative trait loci controlling susceptibility to 11 tumor types were identified. Unsupervised clustering revealed that for several tumor types the same susceptibility loci controlled the cancer outcomes for HZE ions and gamma rays indicating that HZE ions cause the same tumors as gamma rays through the same (or overlapping) pathways in these cases. Survival data also showed that life shortening is greater in females. These findings validate key assumptions of the NASA risk model that females are at greater risk than males and that HZE ion risk can be extrapolated from gamma ray risks. Furthermore, the findings suggest that countermeasures for gamma ray exposures may also be effective against HZE ions. Gamma ray countermeasures are already being actively pursued by other agencies.

# Extending the use of Quantitative Genetics for Space Biology

Besides increased risk of radiogenic cancer, other long-term spaceflight health risks include cardiovascular injury, bone loss, and neurocognitive decrements. Quantitative genetics studies using ground-based rodent models could be used in a similar manner to understand the degree to which each of these risks may be modulated by genetic susceptibility. By defining groups of genetically susceptible and resistant individuals, the effectiveness of countermeasures could also be explored.  The challenge for such approaches will be to develop experimental designs where the statistical power comes from ground-based studies which can be followed up using minimal numbers of mice or rats sent into space to verify the conclusions.  Fortunately, tools exist that make this possible.  What follows is a mock microgravity study that takes advantage of a hybrid approach using ground-based analogs for large-scale screens and flight experiments for validation.

## Mock Study on Bone Strength

As an example, among the knowledge gaps in microgravity effects on bone strength are:

1.       Are preflight bone marrow density (BMD) or other measurements of bone strength or measurements of bone remodeling predictive of bone loss in flight and/or the extent of recovery post-flight? Note that this been examined in flight crews (see Gabel 2020) but by necessity this was a “small N” study and the phenotypes tested were limited to those that could ethically be quantified in humans.

2.       Which physiologic pathways determine the extent of bone loss due to microgravity?

3.       Which pathways determine the extent of recovery postflight?

4.      Are the same pathways used in mice also used in humans?  This is important because initial studies of novel pharmacologic agents would likely be done in mice

Filling these knowledge gaps would allow rational selection of potential pharmacologic and non-pharmacologic countermeasures against loss of bone strength. Also, because quantitative genetics approaches are partly agnostic regarding pre-existing biological knowledge, they will likely lead to novel pathways and the identification of genes that control those pathways.

Developing a study design employing quantitative genetics tools to address these knowledge gaps would begin with mining information on variations of bone mineral density and bone strength in genetically diverse heterogeneous stocks (Al-Barghouti 2021) and inbred strains of mice (<https://phenome.jax.org/>, search term “bone”).  Using approximately 300 male and 300 female DO heterogeneous stock mice Al-Barghouthi et al quantified 55 skeletal phenotypes related to bone strength in 5 categories: geometry, biomechanics, microarchitecture, marrow adiposity and histomorphometry.  These skeletal phenotypes give a more comprehensive view of bone strength than bone mineral density alone.  Cortical bone RNA-seq data were used to identify transcriptional co-expression networks leading to the discovery of 66 genes likely to be causal for bone marrow density loci previously observed in human genome-wide association studies.  In addition, 28 quantitative trait loci controlling bone strength associated traits were identified in the DO mice.

Microgravity and reduced gravity are simulated in ground based rodent studies by hindlimb suspension or partial weight unloading harnesses.  The study described above could be repeated in mice subjected to 30 days of hindlimb unloading followed by 30 days of recovery to identify QTL as well as physiological networks and key genetic variations that determine bone loss and the extent of recovery from it.  Since these studies require longitudinal measures in the same mice only “non-destructive” phenotypes (those that don’t require euthanasia) would be measured at the baseline timepoint.

Studies of this type would identify genetically controlled pathways that determine the extent of bone weakening following unloading and the extent of recovery afterwards.  Some of these pathways are likely to be novel and may be “druggable”, thus pointing to potential countermeasures.

As noted above, hindlimb unloading is only a surrogate for true microgravity.  Validation of the results from the discovery phase of the study described above would require flight experiments.  Current ISS capabilities allow up to 40 mice to be flown at a time. This number is compatible with an experimental design that employs recombinant inbred strains derived from the heterogeneous stock used in the discovery phase.  These strains, known as Collaborative Cross (CC) strains, have been created for the DO heterogeneous stock.  A panel of ten CC strains would be selected to cover the phenotypic range seen in the traits assayed in the discovery phase.  Four mice from each of the 10 CC strains could then be flown to confirm the discovery phase results.  In addition, the selected CC strains could be used to systematically evaluate potential countermeasures.

# Conclusion

While there are presently gaps in our understanding of how genetic background affects adaptation to the spaceflight environment, quantitative genetic approaches with rodent models can be implemented within the logistical constraints of spaceflight experimentation through the combination of ground studies in a “discovery phase" and targeted spaceflight studies in a “validation phase”. While this paper specifically outlines an approach to address questions surrounding bone strength, and similar framework could be utilized to address additional questions.

# References

Al-Barghouthi BM, Mesner LD, Calabrese GM, Brooks D, Tommasini SM, Bouxsein ML, Horowitz MC, Rosen CJ, Nguyen K, Haddox S, Farber EA, Onengut-Gumuscu S, Pomp D, Farber CR. Systems genetics in diversity outbred mice inform BMD GWAS and identify determinants of bone strength. Nat Commun. 2021 Jun 7;12(1):3408. doi: 10.1038/s41467-021-23649-0. PMID: 34099702; PMCID: PMC8184749.

Chitre AS, Polesskaya O, Holl K, Gao J, Cheng R, Bimschleger H, Garcia Martinez A, George T, Gileta AF, Han W, Horvath A, Hughson A, Ishiwari K, King CP, Lamparelli A, Versaggi CL, Martin C, St Pierre CL, Tripi JA, Wang T, Chen H, Flagel SB, Meyer P, Richards J, Robinson TE, Palmer AA, Solberg Woods LC. Genome-Wide Association Study in 3,173 Outbred Rats Identifies Multiple Loci for Body Weight, Adiposity, and Fasting Glucose. Obesity (Silver Spring). 2020 Oct;28(10):1964-1973. doi: 10.1002/oby.22927. Epub 2020 Aug 29. PMID: 32860487; PMCID: PMC7511439.

Edmondson EF, Gatti DM, Ray FA, Garcia EL, Fallgren CM, Kamstock DA, Weil MM. Genomic mapping in outbred mice reveals overlap in genetic susceptibility for HZE ion- and γ-ray-induced tumors. Sci Adv. 2020 Apr 15;6(16):eaax5940. doi: 10.1126/sciadv.aax5940. PMID: 32494593; PMCID: PMC7159905.

Gabel L, Liphardt AM, Hulme PA, Heer M, Zwart SR, Sibonga JD, Smith SM, Boyd SK. Pre-flight exercise and bone metabolism predict unloading-induced bone loss due to spaceflight. Br J Sports Med. 2021 Feb 17:bjsports-2020-103602. doi: 10.1136/bjsports-2020-103602. Epub ahead of print. PMID: 33597120.

Gonzales NM, Seo J, Hernandez Cordero AI, St Pierre CL, Gregory JS, Distler MG, Abney M, Canzar S, Lionikas A, Palmer AA. Genome wide association analysis in a mouse advanced intercross line. Nat Commun. 2018 Dec 4;9(1):5162. doi: 10.1038/s41467-018-07642-8. PMID: 30514929; PMCID: PMC6279738.

Keele GR, Prokop JW, He H, Holl K, Littrell J, Deal A, Francic S, Cui L, Gatti DM, Broman KW, Tschannen M, Tsaih SW, Zagloul M, Kim Y, Baur B, Fox J, Robinson M, Levy S, Flister MJ, Mott R, Valdar W, Solberg Woods LC. Genetic Fine-Mapping and Identification of Candidate Genes and Variants for Adiposity Traits in Outbred Rats. Obesity (Silver Spring). 2018 Jan;26(1):213-222. doi: 10.1002/oby.22075. Epub 2017 Nov 28. PMID: 29193816; PMCID: PMC5740008.

Keele GR, Prokop JW, He H, Holl K, Littrell J, Deal AW, Kim Y, Kyle PB, Attipoe E, Johnson AC, Uhl KL, Sirpilla OL, Jahanbakhsh S, Robinson M, Levy S, Valdar W, Garrett MR, Solberg Woods LC. Sept8/SEPTIN8 involvement in cellular structure and kidney damage is identified by genetic mapping and a novel human tubule hypoxic model. Sci Rep. 2021 Jan 22;11(1):2071. doi: 10.1038/s41598-021-81550-8. PMID: 33483609; PMCID: PMC7822875.

Nicod J, Davies RW, Cai N, Hassett C, Goodstadt L, Cosgrove C, Yee BK, Lionikaite V, McIntyre RE, Remme CA, Lodder EM, Gregory JS, Hough T, Joynson R, Phelps H, Nell B, Rowe C, Wood J, Walling A, Bopp N, Bhomra A, Hernandez-Pliego P, Callebert J, Aspden RM, Talbot NP, Robbins PA, Harrison M, Fray M, Launay JM, Pinto YM, Blizard DA, Bezzina CR, Adams DJ, Franken P, Weaver T, Wells S, Brown SD, Potter PK, Klenerman P, Lionikas A, Mott R, Flint J. Genome-wide association of multiple complex traits in outbred mice by ultra-low-coverage sequencing. Nat Genet. 2016 Aug;48(8):912-8. doi: 10.1038/ng.3595. Epub 2016 Jul 4. PMID: 27376238; PMCID: PMC4966644.

Ouellette AR, Neuner SM, Dumitrescu L, Anderson LC, Gatti DM, Mahoney ER, Bubier JA, Churchill G, Peters L, Huentelman MJ, Herskowitz JH, Yang HS, Smith AN, Reitz C, Kunkle BW, White CC, De Jager PL, Schneider JA, Bennett DA, Seyfried NT; Alzheimer’s Disease Genetics Consortium, Chesler EJ, Hadad N, Hohman TJ, Kaczorowski CC. Cross-Species Analyses Identify Dlgap2 as a Regulator of Age-Related Cognitive Decline and Alzheimer's Dementia. Cell Rep. 2020 Sep 1;32(9):108091. doi: 10.1016/j.celrep.2020.108091. PMID: 32877673; PMCID: PMC7502175.

Parker CC, Gopalakrishnan S, Carbonetto P, Gonzales NM, Leung E, Park YJ, Aryee E, Davis J, Blizard DA, Ackert-Bicknell CL, Lionikas A, Pritchard JK, Palmer AA. Genome-wide association study of behavioral, physiological and gene expression traits in outbred CFW mice. Nat Genet. 2016 Aug;48(8):919-26. doi: 10.1038/ng.3609. Epub 2016 Jul 4. PMID: 27376237; PMCID: PMC4963286.

Rat Genome Sequencing and Mapping Consortium, Baud A, Hermsen R, Guryev V, Stridh P, Graham D, McBride MW, Foroud T, Calderari S, Diez M, Ockinger J, Beyeen AD, Gillett A, Abdelmagid N, Guerreiro-Cacais AO, Jagodic M, Tuncel J, Norin U, Beattie E, Huynh N, Miller WH, Koller DL, Alam I, Falak S, Osborne-Pellegrin M, Martinez-Membrives E, Canete T, Blazquez G, Vicens-Costa E, Mont-Cardona C, Diaz-Moran S, Tobena A, Hummel O, Zelenika D, Saar K, Patone G, Bauerfeind A, Bihoreau MT, Heinig M, Lee YA, Rintisch C, Schulz H, Wheeler DA, Worley KC, Muzny DM, Gibbs RA, Lathrop M, Lansu N, Toonen P, Ruzius FP, de Bruijn E, Hauser H, Adams DJ, Keane T, Atanur SS, Aitman TJ, Flicek P, Malinauskas T, Jones EY, Ekman D, Lopez-Aumatell R, Dominiczak AF, Johannesson M, Holmdahl R, Olsson T, Gauguier D, Hubner N, Fernandez-Teruel A, Cuppen E, Mott R, Flint J. Combined sequence-based and genetic mapping analysis of complex traits in outbred rats. Nat Genet. 2013 Jul;45(7):767-75. doi: 10.1038/ng.2644. Epub 2013 May 26. PMID: 23708188; PMCID: PMC3821058.

Recla JM, Robledo RF, Gatti DM, Bult CJ, Churchill GA, Chesler EJ. Precise genetic mapping and integrative bioinformatics in Diversity Outbred mice reveals Hydin as a novel pain gene. Mamm Genome. 2014 Jun;25(5-6):211-22. doi: 10.1007/s00335-014-9508-0. Epub 2014 Apr 5. PMID: 24700285; PMCID: PMC4032469.

Recla JM, Bubier JA, Gatti DM, Ryan JL, Long KH, Robledo RF, Glidden NC, Hou G, Churchill GA, Maser RS, Zhang ZW, Young EE, Chesler EJ, Bult CJ. Genetic mapping in Diversity Outbred mice identifies a Trpa1 variant influencing late-phase formalin response. Pain. 2019 Aug;160(8):1740-1753. doi: 10.1097/j.pain.0000000000001571. PMID: 31335644; PMCID: PMC6668363.

Saul MC, Philip VM, Reinholdt LG; Center for Systems Neurogenetics of Addiction, Chesler EJ. High-Diversity Mouse Populations for Complex Traits. Trends Genet. 2019 Jul;35(7):501-514. doi: 10.1016/j.tig.2019.04.003. Epub 2019 May 24. PMID: 31133439; PMCID: PMC6571031.

Sittig LJ, Carbonetto P, Engel KA, Krauss KS, Barrios-Camacho CM, Palmer AA. Genetic Background Limits Generalizability of Genotype-Phenotype Relationships. Neuron. 2016 Sep 21;91(6):1253-1259. doi: 10.1016/j.neuron.2016.08.013. Epub 2016 Sep 8. PMID: 27618673; PMCID: PMC5033712.

Solberg Woods LC, Palmer AA. Using Heterogeneous Stocks for Fine-Mapping Genetically Complex Traits. Methods Mol Biol. 2019;2018:233-247. doi: 10.1007/978-1-4939-9581-3\_11. PMID: 31228160.

Tsaih SW, Holl K, Jia S, Kaldunski M, Tschannen M, He H, Andrae JW, Li SH, Stoddard A, Wiederhold A, Parrington J, Ruas da Silva M, Galione A, Meigs J; Meta-Analyses of Glucose and Insulin-Related Traits Consortium (MAGIC) Investigators, Hoffmann RG, Simpson P, Jacob H, Hessner M, Solberg Woods LC. Identification of a novel gene for diabetic traits in rats, mice, and humans. Genetics. 2014 Sep;198(1):17-29. doi: 10.1534/genetics.114.162982. PMID: 25236446; PMCID: PMC4174929.