PROPOSAL

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STATEMENT OF DISCLOSURE PREFERENCE

University of New Hampshire (Organization) in submitting proposal titled Physiology and genomics of dehydration resistance in desert-adapted animals with Matthew D MacManes (Name)

as Principal Investigator/Project Director requires the following procedure be used during its evaluation:

(✔) Permission is hereby granted to evaluate this proposal in accordance with its normal procedures which may include evaluation by reviewers both within and outside the Government.

(☐) Restrict the evaluation of the above proposal to Government reviewers only.

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Cover Page	☒ Yes	☐ No	Statement of Work	☒ Yes	☐ No
Project Abstract	☒ Yes	☐ No	Biographical Sketch	☒ Yes	☐ No
Technical Proposal	☒ Yes	☐ No	Cost Proposal/Budget	☒ Yes	☐ No
Current & Pending Support	☐ Yes	☒ No	Bibliography	☒ Yes	☐ No

Sept 26 2014
Date

Signature of Principal Investigator/Project Director

Date

Signature of person authorized to sign for organization

ARO Form 52A
(Rev. March 2008)

PROJECT ABSTRACT

Every day, soldiers serve in desert environments, forced to endure intense head and aridity. Indeed, these environmental stressors may pose a greater threat than that of enemy combatants. While billions of dollars have been spent on protecting soldiers from bullets, far less attention has been paid to the more insidious threat of heat and dehydration, which may result in cognitive or physical impairment or even death. What if there was a way to significantly enhance the performance and safety of our soldiers by reducing the physiological need for water, particularly in desert environments? While humans and most other mammals are exquisitely sensitive to dehydration, many animals that evolved in deserts are capable of living without ever drinking water. This basic science research proposal aims to understand the genetic and genomic underpinnings of survival without water in a desert-adapted rodent native to the southwest United States.

I will accomplish this goal by conducting a series of experiments on captive desert rodents housed in a chamber designed to replicate the intense heat and aridity of desert environments, while allowing me to manipulate other environmental variables such as water availability and diet. I will measure multiple physiological parameters including serum electrolyte and urine concentration, as well as animal behavior, which will allow me to better understand the unique physiology of these animals. To assay the genomic processes that underlie physiology, I use the techniques of computational genomics. Here, I will uncover the genome wide patterns of gene expression and methylation, allowing me to identify specific genes and pathways that will become future targets for therapeutic intervention.

In summary, this project will result in the elucidation of genetic mechanisms that enable survival in desert environments via the use of a unique mammalian model of dehydration. Long term, we strive to leverage our understanding of dehydration resistance in desert rodents to reduce the physical and cognitive deficits associated with human dehydration, thus increasing soldier performance and safety.

ABSTRACT

Environmental stressors faced by soldiers operating in desert environments represent serious threats to their physical and cognitive performance. Heat and aridity, while dangerous to humans, are not harmful to animals adapted to these conditions. This research proposal aims to characterize the physiologic and genomic mechanisms that enable desert-adapted rodents to survive, ultimately leading to strategies aimed at enhancing soldier safety and performance in desert environments. I will accomplish this goal by conducting a series of experiments on captive desert rodents housed in a desert chamber. I will measure multiple physiological parameters and assay the underlying patterns of gene expression, isoform use, and methylation. This project will result in the elucidation of genetic mechanisms that enable survival and lay the foundation for future work aimed at developing interventions, specifically reducing the untoward effects of dehydration on soldier performance.

INTRODUCTION

Every day, soldiers serve in desert environments, forced to endure intense head and aridity. Indeed, these environmental stressors may pose a greater threat than that of enemy combatants. While billions of dollars have been spent on protecting soldiers from bullets, far less attention has been paid to the more insidious threat of heat and dehydration, which may result in cognitive or physical impairment or even death. What if there was a way to significantly enhance the performance and safety of our soldiers by reducing the physiological need for water, particularly in desert environments? While humans and most other mammals are exquisitely sensitive to dehydration, many animals that evolved in deserts are capable of living without ever drinking water. This basic science research proposal aims to understand the genetic and genomic underpinnings of survival without water in a desert-adapted rodent native to the southwest United States, *Peromyscus eremicus*. Our long-term goal includes developing the ability to recapitulate the phenotype in non-desert adapted mammals, including humans, to significantly enhance soldier safety and performance in desert environments.

The maintenance of water balance in animals is one of the most important physiologic processes, and is a critical component of desert survival. Humans and other mammals are exquisitely sensitive to changes in osmolality, with slight derangement eliciting physiologic compromise. When the loss of water exceeds dietary intake, dehydration - and in extreme cases, death - can occur. Unlike most mammals, animals living in desert habitats are subjected to long periods of extreme heat and intense drought. As a result, desert animals have evolved mechanisms through which physiologic homeostasis is maintained despite severe and prolonged dehydration. **The proposed research uses a novel approach integrating physiology, genomics, and computational**

biology to better understand how animals thrive in what appears to be unsurvivable conditions. This integrative basic science research program will significantly enhance our understanding of the physiologic processes underlying osmoregulation in extreme environments, the critical 1^{st} step in developing therapies that will enhance soldier performance and safety.

Specifically, I propose to study extreme physiologic water conservation and dehydration tolerance using a captive colony of *Peromyscus eremicus* rodents native to the desert southwest United States. These rodent are housed in a specially designed walk-in desert chamber. This chamber simulates the intense heat and aridity of the natural environment, while preserving the ability to manipulate relevant variables (temperature, humidity, water availability) to meet experimental needs. For animals exposed to various experimental treatments (Figure 1, n=20 per treatment), I will collect and analyze physiologic data relevant to hydration status (*e.g.* serum electrolyte levels) and kidney function (*e.g.* BUN, Creatinine). Because water requirements are related to metabolic rates I will collect data related to metabolism using a chamber designed to function in extreme desert conditions. Lastly, though most individuals are functionally anuric, urine will be collected when available and analyzed for specific gravity and osmolality. In addition to the thorough characterization of physiology related to desert survival, I will characterize the genomic changes that underlie changes in physiology to link genotype with phenotype.

BACKGROUND

The study of adaptation, or the process through which animals become fitted to their environment, has intrigued researchers for decades (Darwin, 1859; Fisher, 1930). Only recently have we possessed the ability to study the underlying genomic mechanisms. Currently, researchers interested in understanding the genetics of adaptation are able to ground modern studies on decades of work aimed at understanding the ecological context within which adaptation occurs. One particularly salient example of the connection between studies of ecology, natural history and modern genomics can be found in the study of physiologic adaptation to desert conditions. Here, remarkable physiologic, morphologic (Dickinson et al., 2007; Huntley et al., 1984; Schmidt-Nielsen and Schmidt-Nielsen, 1950; Schmidt-Nielsen, 1952), and behavioral (Nagy, 1994) adaptation has been studied in the context of desert ecology. These studies provide a rich context for the current work, which aims to understand the links between physiology and genomics in rodents able to thrive in amongst the most harsh of conditions on Earth. Ultimately, this work aims to provide interventions that specifically enhance the performance and safety of our troops serving in desert conditions by reducing or eliminating the untoward effects of dehydration.

The genomic processes related to desert survival have yet to be characterized. The few stud-

ies of genetics in this field have focused on the role of single members of the Aquaporin gene family (but see Bartolo and Donald 2007), which are large membrane-bound proteins critically involved in renal water transport (Kwon et al., 2009; Verkman, 2002; Brown et al., 1995; Nielsen and Agre, 1995). These studies have shown that changes in Aquaporin (AQP) protein abundance and expression may be related to water availability (Böselt et al., 2009; Gallardo et al., 2005; Bozinovic et al., 2003). In addition to changes in expression, another study showed that the AQP4 pathway was completely lost in the desert rodent *Dipodomys merriami merriami* (Huang et al., 2001). Despite these studies, we have a limited understanding of the genomics of renal water and solute regulation in desert animals. While AQPs are functionally important, water and solute balance is extraordinarily complex, and therefore single-gene studies are necessarily limited in their purview. A more complete understanding of this phenotype and its mechanistic underpinnings will require a sophisticated genome-level approach, the intended outcome of the proposed research.

APPROACH

Aim 1: To characterize the physiologic and genomic response (differential gene expression, patterns of methylation and isoform use) to extreme water restriction and heat.

To better understand the physiologic and genomic response underlying dehydration resistance, a series of experiments allowing us to understand how differences in temperature, relative humidity, and water availability affect the desert-adapted rodent *Peromyscus eremicus* will be conducted. These experiments are fundamentally a series of environmental manipulations, as described in Figure 1. The experimental design is fully factorial, meaning that the focal experimental parameter (e.g. water availability) will be tested in the context of the full range of other conditions (e.g. humidity, temperature). *Environmental parameters are chosen to match common conditions faced by soldiers operating in desert environments*, while cool/moist conditions effectively represent a less challenging control environment. Animals are exposed to each experimental condition for 14 days. Animal care is standardized between experiments and includes measures to reduce the water content of food and bedding materials. Both of these variables will be dried in a standard desiccation oven to less than 1% water/volume. Twenty individuals per treatment will be included; power analyses suggest that this sample size will allow for detection of statistical support for patterns with small to medium effect sizes. This design will make it possible to tease-apart the physiologic and genomic responses to the various conditions.

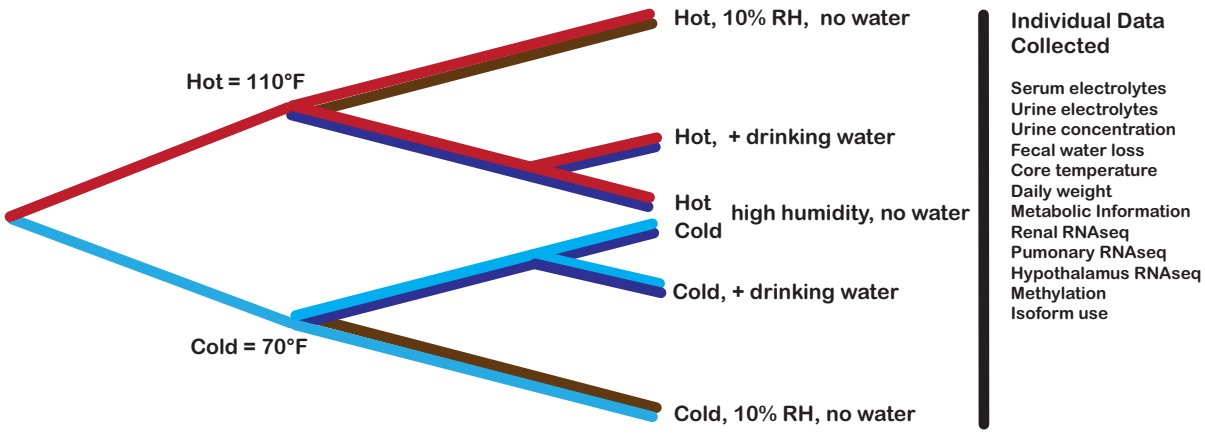

Figure 1: Animals are relegated into either hot or cold treatments. Within treatments (n=20 per treatment), animals are exposed to two weeks of varying levels of aridity, from simulated rainfall where water is available *ad libitum*, to dry, where no water is available. RH=Relative Humidity

For each experiment in Aim 1, physiologic and genomic data will be collected. In the context of limited water intake, how animals achieve electrolyte balance is unknown. Electrolytes are both easy to assay and are critical to physiological well being. Indeed, proper electrolyte balance is fundamental to all other cognitive and physiological processes such as neuronal signal transduction and muscle (including cardiac) contractility. Here, serum electrolytes will be measured using the VetScan VS2 critical care panel, which includes ALT, BUN, Cl, CRE, GLU, K, Na, bicarbonate ion in a 100uL sample volume.

In addition to assaying electrolytes themselves, measures of urine electrolytes and osmolality will be collected, as the urinary system represents the major pathway through which these chemicals are lost. These parameters will be measured using an Atago UG-α urine refractometer and tests conducted at the IDEXX reference lab. Lastly, animals will be weighed to the nearest 0.1gm every other day, including the day of sacrifice. Body temperature will be assayed with weighing using a digital thermometer and probe designed by World Precision Instruments (Sarasota, FL). In connection, feces will be collected and water content will be measured using standard methods.

Key metabolic parameters such as carbon dioxide production and oxygen consumption that may influence water consumption will be collected. In addition, the change in relative humidity within the metabolic chamber will be assayed, which will allow us to understand the rate of pulmonary water loss (or gain). These tests will be measured during a twenty four hour period at the end of the experimental manipulation, just prior to euthanasia, using a metabolic chamber (Sable Inc.) modified for use in the desert chamber. Together, these data will represent a uniquely rich characterization of the physiological state of a desert rodent held in captivity but more importantly,

exposed to conditions typical of the natural environment. All procedures involving vertebrate animals conform to the guidelines provided in (Sikes et al., 2011) and have been approved by the University of New Hampshire Animal Care and Use Committee.

Aim 1a: Determine the physiologic response to drinking-water deprivation, extreme temperature, and humidity in the desert-adapted rodent *P. eremicus*. It is hypothesized that, as a result of unique mechanisms related to solute and water balance, average serum electrolyte concentrations will remain relatively constant throughout various experimental manipulations, however the variance in measured levels between individuals will increase in the most extreme conditions. These differences will be echoed in differences of urine electrolytes and concentration. Predictions regarding other parameters are detailed in Table 1.

Background: The human body consist of 60% water (Jéquier and Constant, 2009). Far from being a static reservoir, proper physiologic function requires water for countless processes including nutrient transport (Haussinger, 1996), signal transduction, pH balance, thermal regulation (Montain et al., 1999), and the removal of metabolic waste. To accomplish these functions, approximately 2 liters of fluid are used daily - these fluids are lost mainly via the gastrointestinal and genitourinary systems, and by evaporative loss, which is accelerated greatly in extremes of heat and aridity (Cheuvront et al., 2010). These losses must be matched by intake (Jéquier and Constant, 2009), mainly in the form of oral fluid intake. Though the body possesses limited reserves, when loss exceeds intake over even a short period of time, dehydration and possibly death can occur. Humans and most other animals are exquisitely sensitive to dehydration and possess limited compensatory mechanisms, particularly when physically active. In contrast, desert rodents often survive in extreme environmental conditions without fluid intake. Understanding the mechanisms underlying this remarkable phenotype requires that we understand the physiology that accompanies it. The work described aims to characterize the physiology of dehydration resistance in desert adapted rodents. This knowledge will allow us to understand the salient differences in renal physiology between humans and desert rodents, ultimately providing a means through which therapies aimed at increasing soldier safety and performance will be developed.

While the prolonged absence of drinking water is invariably fatal for humans and many other animals, one potentially mitigating effect may be the acquisition of water (or limitation of loss) via the pulmonary vasculature, which is known to be variably permeable to water (Berger et al., 2011; Goralski et al., 2010). While pulmonary water acquisition has not been quantified in humans or in mammalian models, the pulmonary vasculature is ideally positioned to retain water from inspired air. Following this, relative humidity, or the amount of extractable water present in respired air, may be important to overall hydration status. The design described above incorporates two differ-

ent levels of humidity to begin to disentangle the effects of drinking water from water acquisition via the pulmonary system.

	Serum E-lytes	Urine Conc.	Fecal Water	Weight
Hot/Dry	Mean Na, Cl, K ↑ to =, Var ↑↑	↑↑↑↑↑	↓↓↓↓↓	↓↓↓↓↓
Hot/Wet				
Rain	Mean Na, Cl, K ↑ to =	↑↑	↓↓	↓↓
Humidity	Mean Na, Cl, K ↑ to =	↑↑↑	↓↓↓	↓↓↓
Cold/Dry	Mean Na, Cl, K ↑ to =, Var ↑↑	↑↑↑↑	↓↓↓↓	↓↓↓↓
Cold				
Rain	Mean Na, Cl, K ↑ to =	↑	↓	↓
Humidity	Mean Na, Cl, K ↑ to =	↑↑	↓↓	↓↓

Table 1: Predicted response given specific experimental manipulations. The number of arrows indicate the predicted relative magnitude of the response.

Although water stress is obviously important to the survival of desert rodents, a phenotype also relevant to human health and wellness, extreme temperatures represent another way in which physiological processes may be challenged. While desert animals may thrive in extreme heat, humans cannot. The physiological response is characterized in model organisms, but not in other animals adapted to these conditions. Genes like heat-shock proteins are protective in humans, but no record of their activity in desert rodents is known.

Research Plan: To accomplish this aim, physiologic data from animals held with and without drinking water will be gathered, factorial with respect to the other conditions (e.g. temperature and humidity). The specific experiments described in Figure 1 will allow us to tease apart the effects of water deprivation from other parameters. Though the data we propose to collect is described above in brief, we plan to collect blood and urine electrolytes and urine specific gravity. We will collect data on fecal water content, animal weight and temperature, and a battery of metabolic parameters. The specific predictions regarding several of these parameters are described in Table 1.

The statistical treatment of the data will include a multivariate regression to establish the relationships between the genomic and physiologic data. Many of these analyses will be conducted with non-parametric tests, as data are often non-normally distributed nor independent. One of the most interesting comparisons will be to understand the relationship between serum sodium and urine sodium, urine concentration, fecal water content, and changes in body weight. Ultimately (e.g. Aim 1b) these data will be linked with patterns of gene expression, methylation, and isoform use to gain a synthetic understanding of dehydration resistance.

Preliminary data: The electrolyte profile of 5 individuals housed at 70F, 50% RH, water *ad lib*, and two individuals housed in identical conditions except that drinking water was withheld has been characterized. Despite being housed in typical laboratory conditions, these animals have remarkably unusual electrolyte panel. For instance, mean serum sodium is 152 mmol/L, chloride is 105 mmol/L, potassium in an un-hemolyzed sample is unusually high by human standards at 8.1 mmol/L, while Creatinine is low, with a mean measurement of 0.25mg/dL, mean blood urea nitrogen (BUN) is 47mg/dL. In contrast, animals without *ad lib* water were obviously dehydrated, with a mean serum sodium of >170 mmol/L and chloride of 126 mmol/L. Severe dehydration was not complicated by renal impairment as evidenced by a mean serum creatinine of 0.3 mg/dL and BUN of 59 mg/dL. The animals kept under these conditions lost a remarkable amount of weight, on average 28% of total body weight. Despite this decline in weight and electrolyte derangement, animals were active as per usual, showing no obvious decline in physical or cognitive function. **These results are shockingly distinct from human response to dehydration and warrant further study.**

Aim 1b: Define patterns of gene expression, isoform use, and methylation given differences in environmental condition. We will understand the genetic response to extreme heat and aridity via a series of Illumina bisulfite and mRNA sequencing and PacBio sequencing experiments, and will link these patterns to individual physiologic state as defined in Aim 1. We hypothesize that genes responsible for water and solute transport will be particularly active in the most extreme conditions in renal and pulmonary tissues, while genes involved in the activation of the hypothalamic-neurohypophysial system will be differentially regulated in the hypothalamus. The genes identified here will provide the requisite foundation on which interventions aimed at increasing soldier safety and performance will be developed.

Background: Broadly speaking, genes underlie the vast majority of observable phenotypes. Whether this relationship is mediated by patterns of expression (e.g. Teets et al. 2012), which itself may be mediated by differences in methylation (Brenet et al., 2011), or by use of alternative splice isoforms (Yukutake and Yasui, 2010), linking genotype to phenotype is extremely difficult. In addition to these mechanisms, function (=phenotype) may be determined by post-translational modifications, such as phosphorylation of specific sites (Moeller et al., 2009). The identification of these mechanisms is important not only because in doing so we gain a deeper understanding of evolution, but also because these molecular mechanisms may be later used as targets for drug development or other therapeutic intervention. With regards to resistance to dehydration, the development of novel interventions is critical, as tens of thousands of troops serve in arid areas - each one of them threatened daily by the dehydration-related decline in physical and cognitive performance.

In model organisms, dehydration precipitates a physiological response that is largely driven by the neuroendocrine system. Very much simplified, the cascade begins with the stimulation of osmoreceptors (Arsenijevic and Baertschi, 1985), which in turn stimulates neurons located in the paraventricular and supraoptic nuclei of the hypothalamus to release anti-diuretic hormone (ADH) (Zingg et al., 1986). ADH then binds to vasopressin-responsive receptors located in the renal medulla, resulting in aquaporin movement to the surface of the collecting duct (Nielsen et al., 1995) which encourages water re-uptake. In addition to the aquaporins, the renin-angiotensin-aldosterone system (Gubler and Antignac, 2009), natriuretic peptides (Totsune et al., 1994), the SLC and mTOR families (Ortells et al., 2012), and potentially other yet to be discovered pathways are important to water balance. Far from canonical, each stage in these cascades is dynamic and therefore pathways revealed in *Mus* and humans may not be equivalent to pathways in uniquely adapted desert animals, particularly given radically different phenotypes. Understanding these mechanisms is critical to the development of interventions.

The genomic processes related to dehydration resistance in desert animals has yet to be characterized. The few studies of genetics that have been conducted have focused on the role of expression of single members of the aquaporin gene family (but see Bartolo and Donald (2007)), which are large membrane-bound proteins that are critically involved in renal water transport (Kwon et al., 2009; Verkman, 2002; Brown et al., 1995; Nielsen and Agre, 1995). These studies have shown that changes in Aquaporin (AQP) protein abundance and expression may be related to water availability (Böselt et al., 2009; Gallardo et al., 2005; Bozinovic et al., 2003). In addition to changes in expression, another study showed that the AQP4 pathway was completely lost in the desert rodent *Dipodomys merriami merriami* (Huang et al., 2001). Despite these studies, we have a limited understanding of the genomics of renal water and solute regulation in desert animals. While AQPs are functionally important, water and solute balance is extraordinarily complex, and therefore single-gene studies are necessarily limited in their purview. A more complete understanding of this phenotype and its mechanistic underpinnings will require a sophisticated genome-level approach, which will be the outcome of the proposed research. In contrast to the limited amount known about patterns of renal gene expression, much less is known about gene expression in other tissues, and absolutely nothing is known about differential methylation or isoform use, though we know that these complexities are mechanistically important to this specific function (Yukutake and Yasui, 2010; Silberstein et al., 2004).

Research Plan: The analysis of the genome wide patterns of response to dehydration will be conducted using the same individuals for which we collected the physiology data. To accomplish this goal, RNAseq libraries for each individual and tissue (n=120 animals * 3 tissues) will

be constructed. Sequencing will be conducted at the New York Genome Center on a HiSeq 2500. We aim to generate approximately 20-30 million 125nt paired-end sequences per sample, which corresponds to 36 high-output HiSeq lanes using 10-way multiplexing.

RNAseq reads derived from kidney, lung, and hypothalamus will be mapped to the existing annotated draft genome that was sequenced using startup funds. This phase of the project will be accomplished using the short read aligner BWA (Li, 2013) and best practices previously established (MacManes, 2014). Differential expression will be evaluated via the Cufflinks package (Trapnell et al., 2012), while evidence for coordinated changes in large numbers of genes will be detected using the software wcgna (Langfelder and Horvath, 2008). The MacManes lab has demonstrated expertise in this area.

Accurate isoform reconstruction is notoriously difficult using high-throughput short read sequence data such as that produced by the Illumina HiSeq platform (Pyrkosz et al., 2013; Hiller et al., 2009), despite the advent of longer read lengths and newer analytical techniques (LeGault and Dewey, 2013; Jiang and Wong, 2009). In projects like this, where differential isoform use may be critical to phenotype, a different approach may be warranted. For instance, the sequencing technology available from Pacific Biosystems (PacBio) is suggested to provide a resolution to isoform reconstruction problems (Au et al., 2013) specifically because it involves a long-read single molecule sequencing strategy (Eid et al., 2009). To identify patterns of differential isoform use, we will sequence poly-A selected mRNA samples using PacBio technology. Reads will be error corrected using the program LSC (Au et al., 2012), and isoforms will be identified using methods contained in Au et al. (2013). Because PacBio throughput is relatively low, which may limit the precision with which quantitation can be achieved, we will explore alternative ways to accurately estimate isoform specific expression. One previously unexplored approach involves estimating expression in the program eXpress (Roberts and Pachter, 2013) using only those reads that map uniquely and unambiguously to a specific isoform. Because this approach is uncharacterized, it will be validated using a set of isoform specific qPCR primers that will allow us to estimate isoform-specific expression using digital PCR.

Lastly, aside from differences in expression of isoform use, patterns of methylation could be important in the development of extreme osmoregulation. Methylation has been shown to be important to many other complex phenotypes including behavior (Lyko et al., 2010), metabolism (Foret et al., 2012), and physiologic stress (including heat stress) response (Sonna et al., 2002). To understand patterns of methylation, a large bisulfite sequence dataset will be generated, which will contain information from every individual included in the mRNAseq experiments as described above. This dataset will allow for the understanding of another layer of genomic complexity not

typically available to researchers conducting RNAseq experiments in isolation. Importantly, in addition to enhancing our understanding of the mechanisms underlying dehydration tolerance, phenotypes related to differential methylation may be prime therapeutic targets.

Preliminary Data: To date, the lab has generated a RNAseq dataset that consists of approximately 30M 150nt SE Illumina reads from the same 5 animals housed in the 'cold/simulated rain' treatment group from which physiology data was collected. We have generated *de novo* transcriptome assemblies as well as mapped mRNA sequencing reads to the reference genome. Though the scope of the analyses is preliminary, the results are interesting. 99.7% of the RNAseq reads map to the genome, with over 73% mapping concordantly. This suggests that the content of the draft genome is compete and that genic contiguity is high. We have recovered many of the aquaporin genes, as well as many other critical genes including vasopressin and its receptor, Renin, Angiotensin, Angiotensin Converting Enzyme, and the genes that code for the natriuretic peptides. We also have estimated expression for all transcripts. Interestingly, within the aquaporin genes, Aquaporins 1 and 2 had the highest expression while expression of Aquaporin 5, 9, 10 and 12 were undetectable, though they are present in the genomic reference.

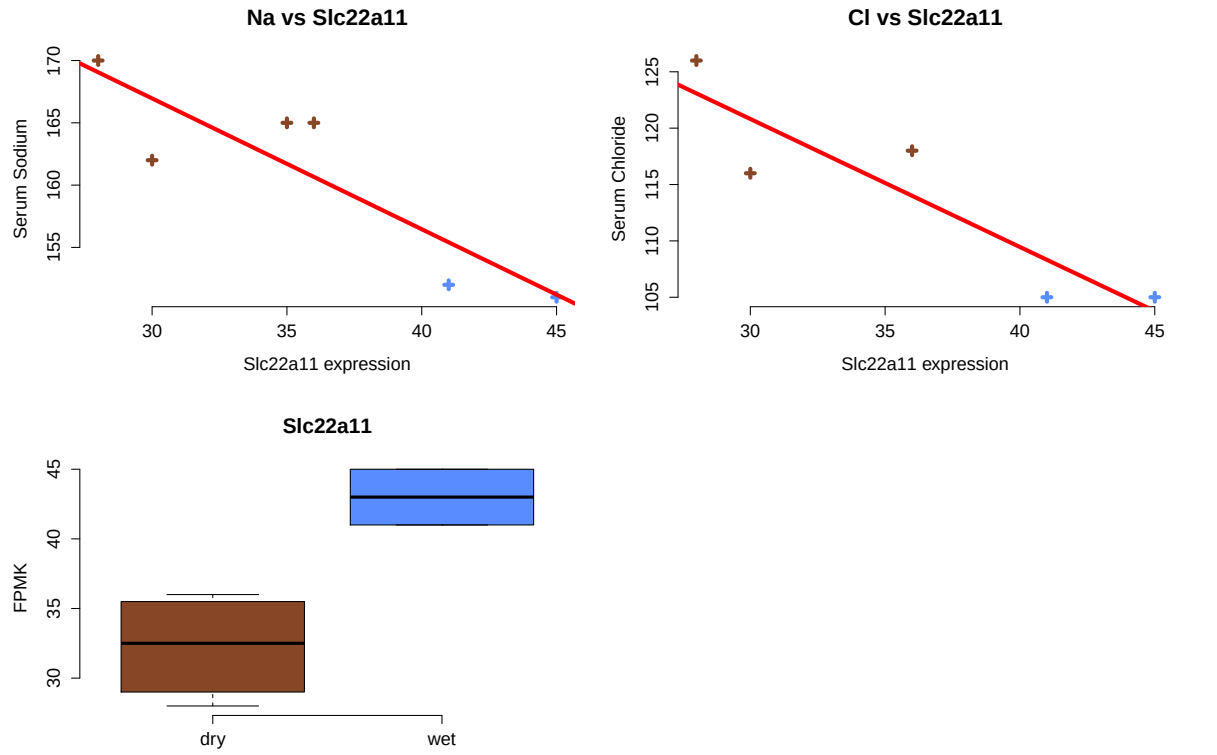

Figure 2: The solute carrier gene Slc22a11 is significantly differentially expressed between dehydrated (represented by brown color) and hydrated (blue color) mice. Additionally, the gene is strongly related to both serum Sodium and Chloride ions.

Testing for differential gene expression between the hydrated and dehydrated mice, though preliminary, were extremely interesting. While we had the *a priori* expectation that the aquaporins would be differentially expressed, many of the solute carrier (SLC) and ion transport genes were significantly upregulated in the dehydrated mice. Figure 2 illustrates this patters, with Slc22a11, a cation/anion transporter (Koepsell and Endou, 2004), significantly differentially expressed and related to serum Sodium and Chloride concentrations. This suggests a possible compensatory mechanism, especially exciting as solute carriers have recently emerged as novel drug targets (Raskandersen et al., 2013). Together, these biological results coupled with increasing interest from pharma suggests that the development of strategies aimed at enhancing soldier safety and performance may ultimately be possible.

Expected Outcome: Upon completion of Aim 1, we will have a synthetic understanding of the physiologic and genomics patterns associated with extreme osmoregulation required for survival in desert environments. These data will allow us to generate a list of genes, genomic regions, isoforms, and methylation states putatively linked to the phenotype of interest. This list is critical, and will form the basis for future applied work, which will propose the development of a system where manipulation of specific genes is possible (e.g. the CRISPR/CAS9 transgenic system or via inhibitory medicines), thus moving the work from correlation to causation. Ultimately, we envision partnering with experts in drug development and delivery to bring these interventions to humans, specifically with the goal of enhancing soldier safety and performance in desert environments.

Aim 2: Given the transition from the obligate intake of fluids as infants to a complete absence later in life, the ontogeny of physiologic water conservation will be elucidated.

Background: Given that desert adapted mice are capable of surviving without water as adults but dependent on liquid intake as neonates, the study of the ontogeny of physiologic water conservation is extremely interesting and relevant to the current work. The study of gene expression in renal, pulmonary and hypothalamus tissue types along the transition from the intrauterine environment through birth in the context of differences in oral fluid intake is remarkably novel and will yield unique insights into physiologic water conservation. Although several studies have assayed renal gene expression in neonates, these studies have typically been limited to a small number of genes in a specific context (e.g. studies of hypertension (Sampson et al., 2012; Shanmugam, 1996)). The proposed work aims to leverage this unique developmental stage to uncover genes underlying this transition. While the physiology of neonates is clearly different from that of soldiers working in desert environments, gaining an understanding of the mechanisms underlying this developmental transition from *ad libitum* water intake to a compete absence of water intake will directly inform our understanding for this work. This aim may wholly reinforce the findings of aim 1 or suggest completely different pathways; both outcomes would clearly enhance the overarching goals of the

research project.

In addition to the transition from lactation-dependence through weaning, an even more fundamental transition happens at birth, which is accompanied by substantial changes in renal physiology. In utero, large volumes of dilute urine are produced (Wintour and Moritz, 1997) while post-birth, relatively small volumes of concentrated urine are typical. While this trend appears to be canonical in well-characterized mammal models, whether the fetuses of water-stressed desert-adapted mice follow this trend is unknown and may be extremely revealing in the context of dehydration resistance. The proposed work aims to use a genome wide approach to characterize these transitions in desert-adapted rodents. This work will provide novel insights into fundamental biological processes, bearing hard upon dehydration resistance as directly relevant to the explicit goals of this project.

Research Plan: This phenomenon will be explored using fetal and neonatal mice whose mothers are exposed to treatments and an abbreviated set of methods listed in Aim 1. Many of the physiological measurements (e.g. blood and urine analyses) will be impossible to collect in very young animals secondary to sample volume requirements, though a full battery of genomic tests will be possible. To evaluate the ontogeny, five fetal and neonatal mice will be culled per treatment at four different time-points (immediately prior to birth, 2 hours after birth, mid-lactation (approximately 10 days after birth), 1 day after weaning). These time-points have been chosen as together they will allow us to assay the breadth of developmental stages. We hypothesize that patterns of gene expression, methylation, and isoform use will resemble those common in conditions where water is available *ad lib*, though the novelty of this aspect of the study limits firm predictions.

Expected Outcome: Upon completion of Aim 2, we will have a synthetic understanding of the genomics patterns associated with the ontogeny of extreme osmoregulation. These data, together with the data associated with Aim 1 will allow us to generate a list of genes, genomic regions, isoforms, and methylation states putatively linked to the phenotype of interest. This list is critical, and will form the basis for future applied work, which will propose the development of a system where manipulation of specific genes is possible (e.g. the CRISPR/CAS9 transgenic system or via inhibitory medicines), thus moving the work from correlation to causation. Ultimately, we envision partnering with experts in drug development and delivery to bring these interventions to humans, here specifically with the goal of enhancing soldier safety and performance in desert environments.

CONCLUSIONS

Soldiers serving in desert environments are forced to endure intense heat and aridity. Indeed,

these environmental stressors represent a real threat to the health and performance of individuals. While humans are generally ill-suited to perform in these environments other mammals, specifically those having evolved in deserts are extremely well adapted. In particular, many desert mammals may survive without water intake. Understanding the physiology and genetics of this remarkable ability will provide the critical 1^{st} steps toward enhancing soldier safety and performance in desert environments by reducing the requirements for oral fluid intake. This basic science research proposal aims to understand the physiology, genetic and genomic underpinnings of survival without water in a desert-adapted rodent native to the Southwest United States, *Peromyscus eremicus*. Our long-term goal includes developing the ability to recapitulate the phenotype in non-desert adapted mammals, including humans.

Table 2: Timeline

Activity	FY2015	FY2016	FY2017
Increase colony size & ID animals for experiments	X		
Conduct physiology experiments -- AIM 1A	X		
Collect & analyze expression data -- AIM 1B	X		
Analyze bisulfite and PacBio data -- AIM 1B		X	
Animal breeding in prep for Aim 2		X	
Collect & analyze genomic data -- AIM 2		X	X
Write papers		X	X
Present results at international conference		X	X
Prepare grant aimed at funding applied research		X	X
Train undergrad, grad students	X	X	X
Disseminate info	X	X	X

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Expertise related to proposed research

The primary goal of the MacManes lab is to understand the genomic underpinnings of complex phenotypes and adaptation. This work integrates the power of novel sequencing technologies with ecology, physiology, and behavior. The lab has recognized authority in genome and transcriptome assembly as a developer of the Trinity and Trinotate software packages, and co-author of the recent Assemblathon 2 paper. In addition, the lab has expertise in the quality control and pre-processing of high-throughput sequencing data, with both published manuscripts and software packages.

Professional Preparation

1996 - 1999 **Associate of Science**. Broome Community College. Binghamton, NY. Major: Nursing

2002 - 2005 **Bachelor of Science.** The University of Michigan - Ann Arbor. Major: Natural Resources Minor: Ecology and Evolutionary Biology

2005 - 2011 **Doctor of Philosophy.** The University of California - Berkeley. Integrative Biology.

2011 - 2013 **Postdoctoral Training.** The University of California, Berkeley. California Institute for Quantitative Biosciences

Appointments

2013 - **Assistant Professor** The University of New Hampshire. Department of Molecular, Cellular & Biomedical Sciences.

2013 - **Faculty Member** Hubbard Center for Genome Studies

Five publications related to the proposed project

2014 **Matthew. D. MacManes** and Michael B. Eisen Characterization of the transcriptome, nucleotide sequence polymorphism, and natural selection in the desert adapted mouse *Peromyscus eremicus* submitted PeerJ. http://dx.doi.org/10.1101/009134.

2014 **Matthew D. MacManes** On optimal trimming of high-throughput sequence data. *Frontiers in Genetics. 5:13*. http://dx.doi.org/10.3389/fgene.2014.00013.

2013 Haas, Brian J., Papanicolaou, Alexie, Yassour, Moran, Grabherr, Manfred, Blood, Philip D., Bowden, Joshua, Couger, Matthew Brian, Eccles, David, Li, Bo, Lieber, Matthias, **MacManes, Matthew D.,** Ott, Michael, Orvis, Joshua, Pochet, Nathalie, Strozzi, Francesco, Weeks, Nathan, Westerman, Rick, William, Thomas, Dewey, Colin N., Henschel, Robert, Leduc, Richard D., Friedman, Nir, Regev, Aviv *De novo* transcript sequence reconstruction from RNA-Seq: reference generation and analysis with Trinity. *Nature Protocols.* 8, 1494 - 1512 http://dx.doi.org/10.1038/nprot.2013.084.

2013 **Matthew. D. MacManes** and Michael .B. Eisen. Improving transcriptome assembly through error correction of high-throughput sequence reads. *PeerJ.* 1:e113 http://dx.doi.org/10.7717/peerj.113.

2013 Bradnam, Keith R., Fass, Joseph N., Alexandrov, Anton, Baranay, Paul, Bechner, Michael, Birol, Inanç, Boisvert, Sébastien, Chapman, Jarrod A., Chapuis, Guillaume, Chikhi, Rayan, Chitsaz, Hamidreza, Chou, Wen-Chi, Corbeil, Jacques, Del Fabbro, Cristian, Docking, T Roderick, Durbin, Richard, Earl, Dent, Emrich, Scott, Fedotov, Pavel, Fonseca, Nuno A., Ganapathy, Ganeshkumar, Gibbs, Richard A., Gnerre, Sante, Godzaridis, Elénie, Goldstein, Steve, Haimel, Matthias, Hall, Giles, Haussler, David, Hiatt, Joseph B., Ho, Isaac Y., Howard, Jason, Hunt, Martin, Jackman, Shaun D., Jaffe, David B., Jarvis, Erich, Jiang, Huaiyang, Kazakov, Sergey, Kersey, Paul J., Kitzman, Jacob O., Knight, James R., Koren, Sergey, Lam, Tak-Wah, Lavenier, Dominique, Laviolette, François, Li, Yingrui, Li, Zhenyu, Liu, Binghang, Liu, Yue, Luo, Ruibang, Maccallum, Iain, **MacManes, Matthew D.,** Maillet, Nicolas, Melnikov, Sergey, Naquin, Delphine, Ning, Zemin, Otto, Thomas D., Paten, Benedict, Paulo, Octávio S., Phillippy, Adam M., Pina-Martins, Francisco, Place, Michael, Przybylski, Dariusz, Qin, Xiang, Qu, Carson, Ribeiro, Filipe J., Richards, Stephen, Rokhsar, Daniel S., Ruby, J Graham, Scalabrin, Simone, Schatz, Michael C., Schwartz, David C., Sergushichev, Alexey, Sharpe, Ted, Shaw, Timothy I., Shendure, Jay, Shi, Yujian, Simpson, Jared T., Song, Henry, Tsarev, Fedor, Vezzi, Francesco, Vicedomini, Riccardo, Vieira, Bruno M., Wang, Jun, Worley, Kim C., Yin, Shuangye, Yiu, Siu-Ming, Yuan, Jianying, Zhang, Guojie, Zhang, Hao, Zhou, Shiguo, Korf, Ian F. Assemblathon 2: genome assembly in three vertebrate species. *GigaScience.* 2:10 http://dx.doi.org/10.1186/2047-217X-2-10.

Other publications

2012 **Matthew D. MacManes** and Eileen A. Lacey. The social brain: Transcriptome assembly and characterization of the hippocampus from a social subterranean rodent, the tuco-tuco (*Ctenomys sociabilis*). *PLOS ONE.* 7(9): e45524 http://dx.plos.org/10.1371/journal.pone.0045524.

2011 Kevin C Rowe, Singhal, Sonal, **MacManes, Matthew D.,** Ayroles, Julien F., Morelli, Toni Lyn, Rubidge, Emily M., Bi Ke, Craig C Moritz Museum genomics: low□cost and high□accuracy genetic data from historical specimens *Molecular Ecology Resources.* 11(6): 1082-1092.

Collaborators

Kelley Thomas, Enrique Lessa, Becca Calisi, Dustin Rubenstein, Kyle Summers, Rasmus Nielsen.

Advisors

Eileen Lacey (PhD supervisor), Michael Eisen (post-doctoral advisor).

Advisees

Lauren Kordonowy, Lindsay Havens, Jennifer Dickson, Kae Lombardo, Kathy Antosca.

CURRENT AND PENDING SUPPORT

The following information should be provided for each investigator and other senior personnel. Failure to provide the information may delay consideration of the proposal.

Investigator: Matthew D MacManes

Support: ☐ Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: Physiology and genomics of dehydration resistance

Source of Support: NSF
Award Amount (or Annual Rate:) $ 264,941 Period Covered: 08/01/15 - 08/01/18
Location of Research: University of New Hampshire
Person-Months Committed to the Project: Cal: Acad: Summer: 1.5

Support: ☐ Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: The Neural Basis of Becoming a Parent: From Genotype to Phenotype

Source of Support: NSF
Award Amount (or Annual Rate:) $ 505,061 Period Covered: 08/01/15 - 08/01/18
Location of Research:
Person-Months Committed to the Project: Cal: Acad: Summer: 1.5

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:
Award Amount (or Annual Rate:) $ Period Covered:
Location of Research:
Person-Months Committed to the Project: Cal: Acad: Summer:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:
Award Amount (or Annual Rate:) $ Period Covered:
Location of Research:
Person-Months Committed to the Project: Cal: Acad: Summer:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:
Award Amount (or Annual Rate:) $ Period Covered:
Location of Research:
Person-Months Committed to the Project: Cal: Acad: Summer:

* If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.

 USE ADDITIONAL SHEETS AS NECESSARY

Facilities and Other Resources:

The MacManes lab currently has several distinct spaces within which research is conducted. PI MacManes has a private office located adjacent to his graduate student offices on the 1st floor of Rudman Hall. This office space is equipped with a desktop computer, printer, telephone and hard-wired internet. Also on the 1st floor is MacManes's molecular lab, which is a 6 bay fully functional space in which graduate student, postdocs and research staff may conduct experiments. This lab is equipped with pipettes, centrifuges, vortexers, heat blocks, refrigerator, freezer, -80 freezer, etc. In addition to these things, there are several conference rooms on each level of the building for conducting meetings and interacting with students. The MacManes lab also owns a large Linux workstation that is equipped with a 64 processors, 512 GB RAM, and a 40Tb Raid5 disk array.

PI MacManes has dedicated animal care space, in which exists the desert chamber, measuring 14ft * 8ft. The temperature in this space can be programmed to reach a maximum of 110F at 10% RH. The controls allow temperature, humidity and lighting to cycle through a typical diurnal cycle. The mouse colony house in this facility consists of 35 mice, a size which can be easily augmented to meet experimental requirements.

A rich collegial culture that exists at UNH and in particular in the department of Molecular, Cellular, and Biomedical Sciences, to which I am a member, that allows me to interact both formally and informally with more experiences colleagues. Further, the college has implemented a plan to limit my teaching and service commitments pre-tenure, which allows me to focus efforts on developing an active and vibrant research program. Beyond the college, I have formed, and will continue to develop relationships with faculty in the department of Computer Science (e.g. Phil Hatcher and Dan Bergeron). At an institutional level, there are numerous systems that aim to support early stage investigators including the Research and Engagement Academy, Up-2-NIH, the Writing Academy, editing services coordinated through the office of the Vice Provost Nisbet. Taken together, these resources provide for an extremely stimulating academic environment.

SHARED RESOURCES

Hubbard Center for Genome Studies (HCGS)
W. Kelley Thomas-Director

The HCGS, located on the 4th floor of Gregg Hall, currently has three research groups which focus on Genomics, Proteomics, and Glycomics. Available bench space will accommodate 28 scientists with additional areas for equipment. The laboratory also incorporates 3 fume hoods, two biosafety cabinets, two environmental rooms and an autoclave. Eight faculty/postdoc offices and cubicles for 13 graduate students are located adjacent to the lab. A penthouse space wired with emergency power will accommodate up to 25 ultracold freezers. All other equipment is located on the same floor of Gregg Hall as the laboratory space. A wide variety of services are provided by the HCGS including sample preparation, Sanger sequencing, and high-throughput sequencing using the HiSeq2500 and associated quality control assays are provided on a recharge basis.

Research Computing and Instrumentation Core Facility (RCI/UNH)
Patrick Messer-Director

RCI's research computing facilities are housed in UNH's Morse Hall, also a designated 10Gbps Science DMZ node, and staffed by sixteen IT professionals specializing in systems and network administration, security, database administration and software/applications engineering.

The facilities include the 2,000 square foot, energy efficient Lenharth Data Center, offering High-Performance Computing (HPC) and networking in support of the UNH research enterprise. The Lenharth Data Center was renovated in 2011 to provide state-of-the art security, power and cooling for over 350 physical computers, networking hardware and over 750 Terabytes of storage. The Lenharth Data Center hosts compute and storage hardware supporting the science performed on the Illumina 2500 High-Throughput DNA Sequencer purchased and installed in 2012 (NSF MRI# DBI-1229361) and located in Gregg Hall. The Lenharth Data Center also houses a Cray XE6m Supercomputer installed in 2013 (NSF MRI# PHY-1229408).

The University Instrumentation Center operates and supports numerous instruments for Nuclear Resonance Spectroscopy, Ultraviolet-Visible-Near-Infrared Spectrophotometry, Infrared Spectrometry, X-ray Photoelectron Spectroscopy, Scanning Electron Microscopy, Transmission Electron Microscopy, Confocal Microscopy, and Energy Dispersive Spectroscopy. The RCI works closely with the Hubbard Center for Genome Studies for managing and disseminating the large datasets generated by next-generation sequencing platforms. These services are provided on a recharge basis.

Rudman Hall Bioinformatics Core Facility

The Rudman Hall Bioinformatics Core Facility is a newly developed facility located on the 3rd floor of Rudman Hall, specifically designed to aid researchers (including ESI researchers and their lab members) in developing analytical pipelines for the analysis of genomic data. The facility has work space for 6 researchers and is equipped with a large genomics workstation, as well as several terminals for the remote access of on- and off-campus computer resources. The facility is staffed 50% of the time by a PhD bioinformatician employed by the HCGS.

Institutional Investment

The PI is considered an Early Stage Investigator. UNH offers several support programs to assistant professors, and the PI's research team has taken part in many.

- **Faculty Mentoring Program, a program of the Provost's Office,** is designed to assist junior faculty in their academic career development through the guidance and support of experienced faculty members who serve as role models, advisors, and mentors. Interactive group mentoring sessions provide opportunities for pre-tenured faculty to discuss issues with senior faculty, administrators, and peers, and to exchange ideas, pose questions, and to address concerns about navigating the UNH promotion and tenure process.
- **Faculty Development Grants support professional development of faculty by providing funding for the acquisition of new skills. These funds are intended to support travel or training fees associated with training courses or specialized conferences to advance the careers of junior faculty members.**
- Office of the Senior Vice Provost for Research provides programs throughout the year for new faculty to support advancement of their research and scholarship and introduce them to potential collaborators at UNH. This Office provides research initiation funding and contributes to start-up packages for new faculty.
- UNH Research and Engagement Academy is a faculty development learning community designed to enhance faculty members' scholarly careers by strengthening the quality and quantity of proposals submitted to external funders and increase the diversity of faculty who are awarded grant funding. Each participating scholar works with a scholarly coach, an experienced UNH faculty member. The co-PI participated in this Academy in 2014, working with Prof. Kelley Thomas as a scholarly coach.