Developing Microbial Biomarkers to Non-invasively Assess Health in Wildlife Populations

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Introduction: The composition of the intestinal bacterial community (intestinal microbiome) of mammals is associated with changes in diet, stress, disease and physical condition of the animal[1]. The use of microbiomes as a diagnostic for health has been extensively applied in humans and mice[2]; this provides strong support for its utility in wildlife. When managing threatened and endangered species or game animals, federal and state agencies currently must rely on invasive sampling for reliable health data[3]. Through collaboration and refinement of this concept with Dr. William Holben and colleagues at the University of Montana (UM), we propose the development of microbial biomarkers as a tool for obtaining health data non-invasively in wildlife. Recent exploration into elk (*C. elephus*) rumen bacterial communities provides support for a "core microbiome" shared by elk, but that research did not focus on variation in bacterial taxa at finer scales of taxonomic resolution and its potential to inform correlates of environmental stress acting on the host animal[4]. Ongoing research I am conducting on 16S small subunit rRNA gene-based (hereafter 16S-based) microbial biomarkers in elk shows promise for an individual elk's microbiome composition to distinguish between and predict states of health as it does in humans.

Hypothesis: *If elk fecal microbiomes are associated with individual body condition at high taxonomic resolution, then consistent presence and abundance (or for that matter absence) of specific bacterial taxa in scat will reliably predict states of health and disease in individuals.*

By discovering microbiome characteristics that vary as a function of elk body condition and disease, managers will have the immediate benefit of seeing direct impacts of current and often cryptic environmental stressors on elk populations. This is a low cost, non-invasive sampling method based simply on fecal pellet collection in the field and intestinal microbiome analysis in the lab. The biomarker methodology, once verified, can then be expanded and applied more broadly to include recovering endangered species, a goal supported by major US legislation including the Endangered Species Act[5]. I plan to develop and use microbial biomarkers in tandem with restriction site associated DNA sequencing (RADseq) of extracted host genomic DNA that will also be present in the fecal pellets[6]. These are both important sources of information for supporting sustainability in non-model species with limited existing genomic data[7]. Combining these two approaches we will begin to fill important gaps in our knowledge by providing difficult to measure impacts of environment and disease acting on individuals along with deep insight into population genetic structure, local adaptation, heterozygosity and genomic regions under selection at the population level.

AIM 1: My study will analyze scat samples from wild elk of known body condition within Montana along with GIS collar tracking to make the first step in establishing methods for deep non-invasive monitoring of wild populations, building on this potential from my ongoing original microbial biomarker research.

AIM 2: We will develop the potential for expanded microbiome biomarker use in a diverse range of wildlife species combined with RADseq genomics for deep monitoring and recovery of individual's genetic data for use in species conservation, providing insights and novel solutions to current wildlife management issues.

Methods: Microbial biomarkers will be identified by next generation sequencing of 16S rRNA gene amplicons using the V4 & V5 variable regions in the bacterial rRNA gene to both select for bacteria in a fecal sample through 16S-specific barcoded primer PCR and distinguish between operational taxonomic units (OTUs) once amplicons are sequenced[2]. Collection of scat samples, body condition metrics and GIS collaring of elk will be conducted by an ongoing collaboration

already developed with Montana Fish, Wildlife and Parks (MTFWP) over the next 3 years. This sampling will use currently available and accepted invasive methods[3]. This invasive health data is initially necessary for training and corroborating the predictive ability of our microbial biomarker algorithms (functionally acting as a control) as well as to facilitate the link between habitat use and health through GIS tracking as we move beyond invasive methodologies. This study will allow insights into microbial biomarker stability or change in this system while providing insights into genetic trends among and between populations. Invasive health and non-invasive fecal pellet collection will be performed from individual elk across Montana including the Bitterroot and Sapphire mountains, hunting district 311 and the Tobacco Root Mountains as well as at additional similar sites, but where no invasive sampling will be done.

Metagenomic and 16S studies produce large amounts of data because of the need to sample microbial communities as deeply and completely as possible, but not all OTUs have predictive power during statistical analysis for determining health or disease states of the host. A feature selection algorithm can reduce the dimensionality of the data to provide a more tractable computation of the biological associations between remaining OTUs and disease states. My sequence data will be initially analyzed using the QIIME package[8] followed by a floating search feature selection classifier developed in collaboration with colleagues in the Computer Science Department at UM. I am currently using this approach in a pilot study to define important bacterial taxa within 110 elk microbiomes from the populations above with *a priori* health data. I predict that we will be able to accurately predict and cluster between host health, disease states, geographic location and sex from which the selected OTUs originated defining a highly valuable microbial biomarker methodology worthy of further pursuit.

Summary and Broader Impacts: Elk metagenomic composition has been proposed to contain reliable biomarkers. If these can be further developed and extrapolated with high precision, it will be possible to non-invasively monitor the presence and effects of forms of both observable and cryptic disturbance on the landscape, allowing managers to use this approach as an early warning system for demographic responses to environmental pressures. An added benefit of biomarkers is that they are a cheaper, less invasive alternative for acquiring information on wildlife populations. By integrating the use of population genetics and microbial biomarkers from the same sample source, a holistic management solution is produced for current and long-term trends while maintaining a low sampling effort. I expect that this approach for identifying microbial biomarkers within the fecal microbiome and the bioinformatics techniques used for their analysis will be more broadly applied to the management and conservation of other wildlife species (incl. non-mammals) that will allow federally designated threatened and endangered species to be studied with no perturbation. I hope to continue development of these techniques in collaboration with state and federal managers and incorporate an undergraduate mentoring program, continuing a tradition of mentorship in the Holben Lab that includes a prior HHMI award and NSF-REU site awards on which Holben was PI. This will allow undergraduates from diverse backgrounds to participate in a highly collaborative environment with professionals from both government and academia on leading edge research that can act as a springboard for producing competitive STEM scientists while achieving wildlife conservation goals.

References: 1. Hooper, L. V. *et al. Science* 336 (2012); Cho, I. and M. J. Blaser, *Nature Reviews Genetics* 13 (2012); **2**. Segata, N. et al. *Genome Biol.* 12 (2011); **3.** Cook, R. C. *et al. Journal of Wildlife Management* 65 (2001); **4.** Gruninger, R. J. *et al. PloS one* 9 (2014); **5.** ESA, 16 USC Sec. 1531. Print (1973); **6.** Davey, J.W, and M. L. Blaxter *Briefings in Functional Genomics* 9 (2010); **7.** Davey, J. W. *et al. Nature Reviews Genetics* 12 (2011); **8.** Caporaso, J. G. *et al. Nature methods* 7 (2010).