Genetic Sex Ratio Analysis of the American Pika (*Ochotona princeps*) in the Rocky Mountain Region

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ABSTRACT

Sex ratios (the ratios of female to male individuals in populations) provide valuable estimates of the health of populations. In species that are territorial and occur in small, isolated populations, biased sex ratios can lead to increased competition among individuals and decrease the genetic diversity of future generations. Having fewer mating opportunities results in the majority of offspring in the next generation coming from only a small number of parents, thus increasing the relatedness of individuals. The American pika (*Ochotona princeps*) is a small, alpine mammal that is predicted to experience habitat range contractions due to climate change. As this occurs, the range will also become more fragmented, decreasing the connectivity of populations. Because of this, understanding which populations are most vulnerable is of utmost importance. Sex ratios are key indicators of a population's potential persistence and, therefore, a part of this understanding. Species that lack significant sexual dimorphism, such as the American pika, can be difficult to sex in the field, requiring a need for genetic based sexing techniques. Chromosomal genetic sexing techniques have been developed using non-invasive hair sampling. In this two part study, we first expanded the use of this method to include fecal samples, performing pairwise comparisons of 13 individuals who were sexed using extractions from both hair and fecal samples to ensure accuracy. Second, we sexed 84 pikas from either fecal or hair samples obtained from three metapopulations in the Colorado and Montana Rocky Mountains. Sex ratios for each population were calculated. We did not observed significant sex ratio bias in any of the metapopulations.

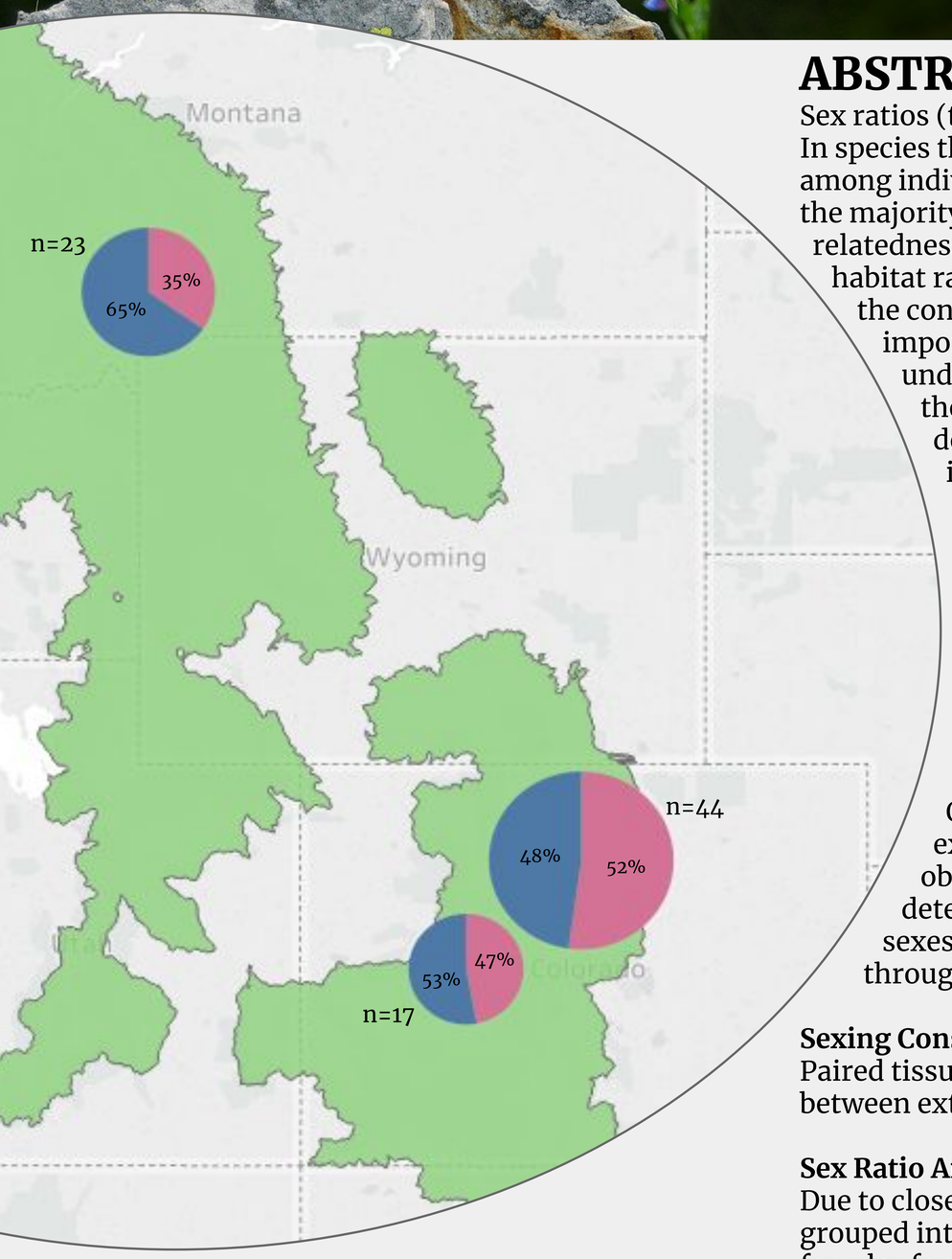

Figure 1. Sex ratios of the three metapopulations in Colorado and Montana. Pie charts scaled to reflect population size

METHODS

A total of 85 tissue samples and 66 fecal samples (not all from unique individuals) were collected by faculty at the University of Colorado - Boulder from 10 sites located in western Colorado and Montana. Using the Qiagen DNeasy Blood & Tissue Kit and Qiagen QIAamp Fast DNA Stool Kit, we extracted DNA from the samples. Extraction concentrations and purities were observed using a NanoDrop spectrophotometer. We used two primer sets to determine the sex of the pika: Ocp10- autosomal microsatellite present in both sexes, SRY- male specific region (Lamb et al. 2014). Amplified products were run through a 2% agarose gel and then visualized under ultraviolet light.

Sexing Consistency Analysis:
Paired tissue and fecal samples (n=13) were used to perform a pairwise comparison between extraction techniques to ensure consistent sexing results.

Sex Ratio Analysis:
Due to close proximity and potential dispersal between many sites, sites were grouped into three larger metapopulations. We totaled the number of males and females for each population and compared the observed sex ratio to the expected 50/50 ratio using a binomial test.

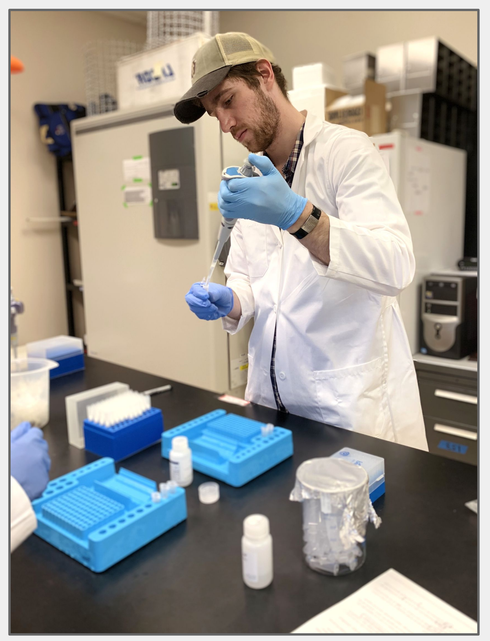

Figure 2. Performing DNA extractions

RESULTS

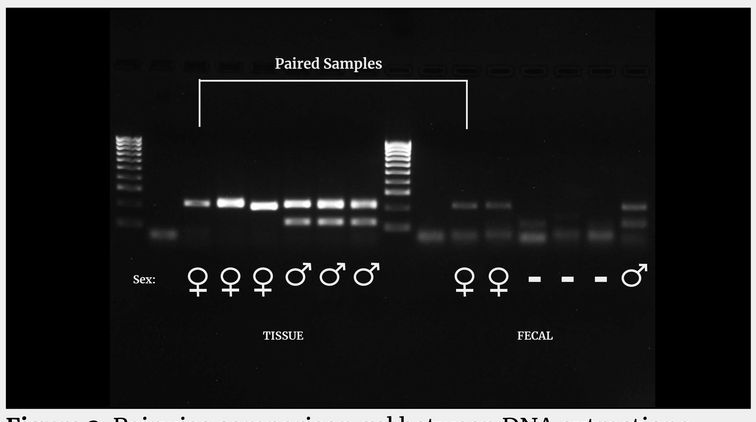

Figure 3. Pairwise comparison gel between DNA extractions from tissue and fecal samples. Extractions from tissue samples produced brighter bands. 100% of tissue extractions and 60% of fecal extractions successfully amplified. Of those successes, all paired samples consistently sexed pikas

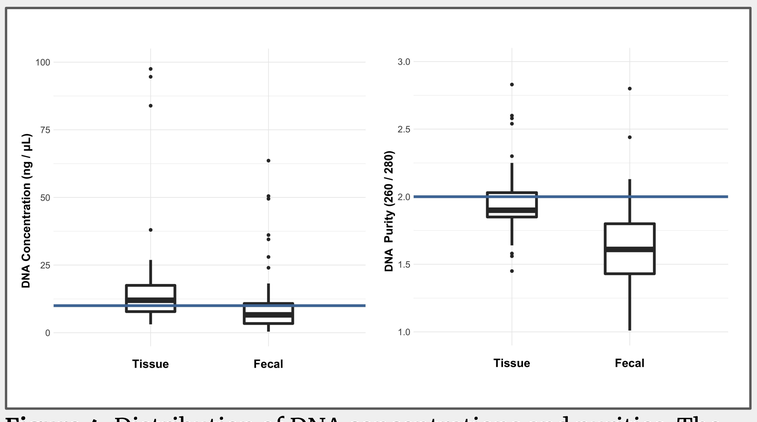

Figure 4. Distribution of DNA concentrations and purities. The blue line indicates expected results from the extraction protocol (Concentration: 10 ng/µL, Purity: 2.0). Seven samples with concentrations greater than 100 ng/µL have been excluded from the plots for clarity

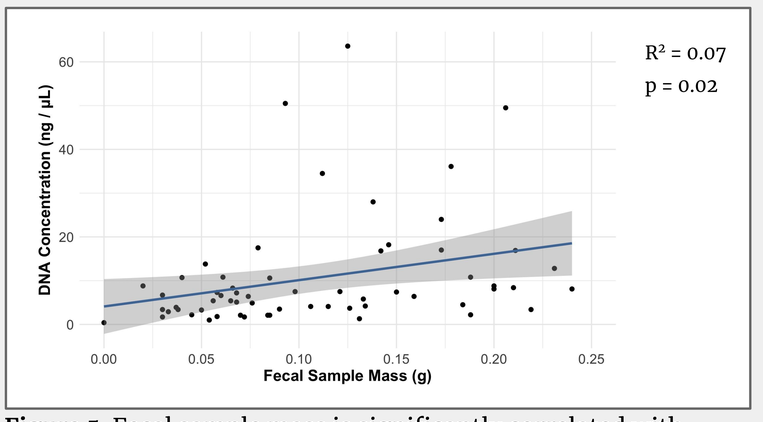

Figure 5. Fecal sample mass is significantly correlated with DNA concentration of the extraction. DNA concentration can be an inconsistent measure of extraction success due to non-pika DNA present in extractions from fecal samples, such as plants

References:
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