Conservation of Sagebrush Ecosystems Through Diet Analysis of an Obligate Species

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OBJECTIVES

- Create high-throughput plant DNA isolation protocol for grouse fecal samples
- Test Primer sets targeting ITS2 gene
- Verify Primer sets with known plant DNA
- Create protocol for PCR of extracted fecal plant DNA
- Create plant DNA barcode library for Next Generation sequencing
- Analyze data to identify plant species

BACKGROUND

Greater Sage Grouse (*Centrocercus urophasianus*) and Sharp-tailed Grouse (*Tympanuchus phasianellus*) are obligate species that depend on sagebrush to survive and serve as an indicator species and umbrella species for the sagebrush ecosystem. The sagebrush ecosystem has been declining rapidly, therefore understanding the impacts on Sage Grouse and Sharp-tailed Grouse could aid researchers and land managers in best practices to protect the long-term viability of the species, the ecosystem, and 350 other species that depend on it, including humans. One way to understand these impacts is through dietary indicators, such as the availability of preferred forage plants. In the past, this has been done by direct observation, which requires many hours in the field, and crop dissection which involves collecting carcasses. These are both time-consuming and costly. Recent advances have shown that diet can be more easily and accurately determined through the sequencing of plant DNA in fecal samples targeting the ITS2 gene of plants.

Sharp-tailed Grouse

Greater Sage Grouse

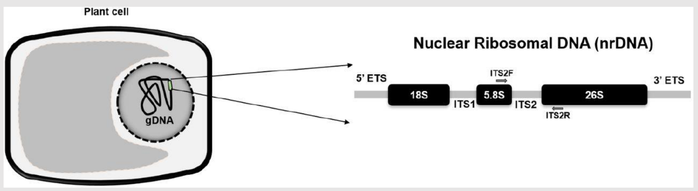

Figure 1: The internal spacer (ITS) region contains the ITS2 gene which can be PCR amplified for plant species identification.

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METHODS AND RESULTS

High-throughput DNA Isolation

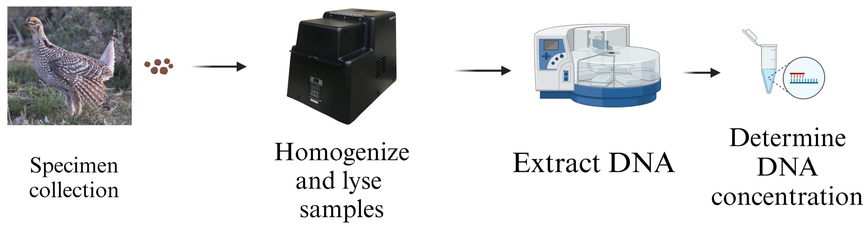
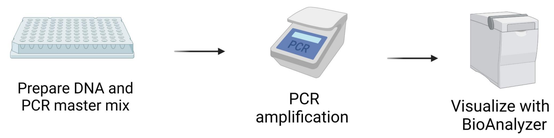


Figure 2: Plant DNA was obtained from grouse fecal sample using the Applied Biosystems MagMAX Plant DNA kit, homogenizing for 1, 2, and 5 mins using a Biospec Beadbeater, and extracted using the KingFisher Flex.

Bead Beater Time	ng/µl	Total ng DNA
1 min	9.4	470
2 mins	12.4	620
5 mins	13.9	695

Table 1. Plant DNA isolated using three different times on the beadbeater. Five minutes will be used going forward.

PCR amplification of ITS2 gene



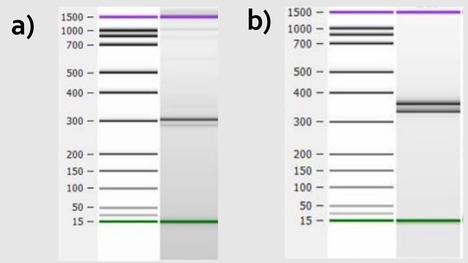
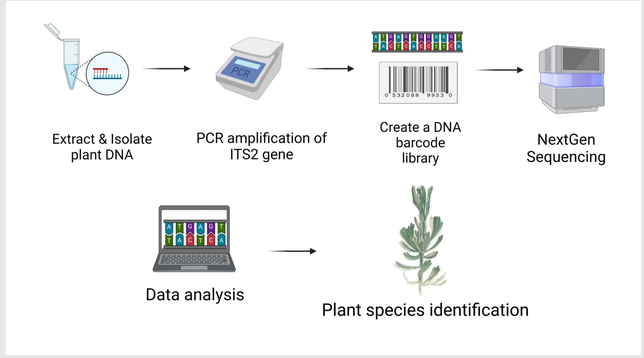


Figure 3 a) primer validation with pure plant DNA b) primer testing in plant DNA pool isolated from grouse fecal DNA

CONCLUSIONS

- Optimized a high-throughput DNA isolation protocol
- Identified ITS2 primers able to amplify plant and fecal DNA
- Optimized a PCR protocol for amplification of desired target

NEXT STEPS



DNA isolation and PCR protocol developed will be used on full sample set

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