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Haemosporidian parasite infections in grouse and ptarmigan: Prevalence and genetic diversity of blood parasites in resident Alaskan birds

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ABSTRACT

Projections related to future climate warming indicate the potential for an increase in the distribution and prevalence of blood parasites in northern regions. However, baseline data are lacking for resident avian host species in Alaska. Grouse and ptarmigan occupy a diverse range of habitat types throughout the northern hemisphere and are among the most well-known and important native game birds in North America. Information regarding the prevalence and diversity of haemosporidian parasites in tetraonid species is limited, with few recent studies and an almost complete lack of genetic data. To better understand the genetic diversity of haemosporidian parasites in Alaskan tetraonids and to determine current patterns of geographic range and host specificity, we used molecular methods to screen 459 tissue samples collected from grouse and ptarmigan species across multiple regions of Alaska for infection by *Leucocytozoon, Haemoproteus,* and *Plasmodium* blood parasites. Infections were detected in 342 individuals, with overall apparent prevalence of 53% for *Leucocytozoon*, 21% for *Haemoproteus*, and 9% for *Plasmodium*. Parasite prevalence varied by region, with different patterns observed between species groups (grouse versus ptarmigan). *Leucocytozoon* was more common in ptarmigan, whereas *Haemoproteus* was more common in grouse. We detected *Plasmodium* infections in grouse only. Analysis of haemosporidian mitochondrial DNA cytochrome *b* sequences revealed 23 unique parasite haplotypes, several of which were identical to lineages previously detected in other avian hosts. Phylogenetic analysis showed close relationships between haplotypes from our study and those identified in Alaskan waterfowl for *Haemoproteus* and *Plasmodium* parasites. In contrast, *Leucocytozoon* lineages were structured strongly by host family. Our results provide some of the first genetic data for haemosporidians in grouse and ptarmigan species, and provide an initial baseline on the prevalence and diversity of blood parasites in a group of northern host species.

1. Introduction

Avian haemosporidians belong to a diverse group of vector-borne, protozoan blood parasites that infect a broad range of avian species throughout North America and around the world (Valkiūnas, 2005). Representative species from the genera *Haemoproteus, Plasmodium*, and *Leucocytozoon* have been detected on every continent except Antarctica (Beadell et al., 2009), infecting both wild and domestic birds. Infections by these parasites can vary among taxa and across habitat types (Greiner et al., 1975; White et al., 1978; Durrant et al., 2006), and prevalence in avian host populations may be determined, at least in part, by exposure to viable dipteran vectors (Bennett et al., 1992). Other factors such as age, host suitability, and a multitude of environmental variables may also be important determinants of parasite infection (Martínez-Abraín et al., 2004).

Rapid warming in northern regions has raised concerns about environmentally-driven changes in the distribution and prevalence of disease in birds and other wildlife (Van Hemert et al., 2014). Many vector-borne parasites, including *Plasmodium* and other haemosporidians, are temperature-sensitive and are projected to expand in response to climate warming in the Arctic (Loiseau et al.,

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2012; Altizer et al., 2013). Heavy infection by haemosporidian parasites can cause mild to severe pathogenic effects in various avian species (Ots and Hõrak, 1998; Palinauskas et al., 2008) and exposure of naïve hosts to blood parasites may result in dramatic population-level consequences, such as have occurred among native birds in Hawaii (LaPointe et al., 2012). Recent studies have proposed an increase in haemosporidian infections, particularly *Plasmodium*, among Alaskan bird populations as a result of shifts in temperature, vegetation cover, and vector populations (Loiseau et al., 2012; Oakgrove et al., 2014). However, limited baseline data currently exist for many avian species, particularly year-round residents, making it difficult to assess potential risks and evaluate future patterns of change.

Species of birds from the order Galliformes (Family Phasianidae) have adapted to the cold climates of arctic and sub-arctic regions and inhabit a broad range of habitats across the northern hemisphere (Aldrich, 1963; Braun and Willers, 1967). Given their status as year-round residents, grouse and ptarmigan (Subfamily Tetraonidae) provide excellent model species for studying the prevalence and diversity of locally-transmitted blood parasites in Alaska as well as the relationships between climate variables and parasite infection.

Four species of grouse and three species of ptarmigan occur throughout Alaska's many biomes and each plays an important role as a prey species for predators and game species for subsistence and recreational hunters. All species of tetraonids in Alaska are year-round residents and many of their populations overlap geographically, but occupy vastly different habitats. Spruce Grouse (*Falcipennis canadensis*) and Ruffed Grouse (*Bonasa umbellus*) are typically associated with boreal and mixed deciduous forests, respectively, whereas Rock Ptarmigan (*Lagopus muta*), Willow Ptarmigan (*Lagopus lagopus*), and White-tailed Ptarmigan (*Lagopus leucura*) inhabit more sparsely vegetated arctic and subarctic tundra and subalpine environments (Aldrich, 1963). Sooty Grouse (*Dendrogapus fuligninosus)* populations in Alaska are found in the temperate coastal rainforests of southeastern Alaska and Sharp-tailed Grouse (*Tympanuchus phasianellus*) tend to prefer open grassy habitat with shrub-like brush in interior Alaska (Aldrich, 1963; Dublin and Taras, 2005). Grouse and ptarmigan populations are secondary prey species to many predators and undergo cyclical population fluctuations (Holmstad et al., 2005). Some researchers have hypothesized that parasitic infection may contribute to these population fluctuations, either as a direct cause of mortality or via a secondary effect such as decreasing mobility or delayed flushing response (Fallis, 1945; Holmstad et al., 2006; Skirnisson et al., 2012).

Early microscopic studies from North America determined that most tetraonid species were infected by at least one genus of haemosporidian parasite, with many individuals showing diverse infections of multiple parasite genera at moderate to high prevalence (Fallis, 1945; Stabler et al., 1967a,b; Bennett and Inder, 1972; Mahrt, 1981; Forbes et al., 1994). Although research on haemosporidian infections in Alaskan tetraonids has been extremely limited, two historical studies focused on grouse and ptarmigan species within specific regions of the state. Stabler et al. (1967a) sampled Rock Ptarmigan in interior Alaska using microscopy and found 88% prevalence of *Leucocytozoon* parasites and no evidence of *Haemoproteus* or *Plasmodium* infection. Additionally, Stabler et al. (1967b) collected blood smears from Spruce Grouse in the southwestern and southcentral regions of Alaska, where they detected 80% and 60% prevalence of *Leucocytozoon* and *Haemoproteus,* respectively, and no *Plasmodium*. No contemporary studies of blood parasite prevalence in Alaskan tetraonids have been conducted. Molecular-based detection methods have become more widely used in the past decade, allowing for detailed description of parasite genetic diversity, including identification of unique parasite lineages in each genus (Bensch et al., 2000; Hellgren et al., 2004 Waldenström et al., 2004). Since the advent of these methods, however, there has been little research on the genetic diversity of haemosporidians in grouse and ptarmigan worldwide (Sato et al., 2007), and none in Alaska.

To address the lack of current knowledge about blood parasite infection in grouse and ptarmigan species in Alaska and to provide information on the genetic diversity of parasite lineages that infect this group of resident birds, our objectives for this study were to: (1) to estimate the prevalence of haemosporidian infections (genera *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*) in grouse and ptarmigan species throughout the state of Alaska; (2) obtain genetic information to estimate the diversity of identified *Haemoproteus, Plasmodium*, and *Leucoctyzoon* parasite lineages; and (3) evaluate the relationship between prevalence of blood parasite infection and geographic region, age, and species of the host. Our results will provide some of the first genetic data on haemosporidian lineages infecting tetraonid species and add new information about blood parasite prevalence and distribution in resident avian hosts from northern regions.

2. Materials and methods

2.1. Sample collection

Wings from hunter-harvested grouse and ptarmigan were voluntarily submitted to the Alaska Department of Fish and Game through their grouse and ptarmigan wing collection program from October 2012 to November 2014. We received 459 samples from four species of grouse (Ruffed Grouse, Spruce Grouse, Sharp-tailed Grouse, and Sooty Grouse) and three species of ptarmigan (Willow Ptarmigan, Rock Ptarmigan, and White-tailed Ptarmigan) from multiple geographic regions of Alaska (Fig. 1). All collections occurred between August and April, primarily during the non-breeding season. Wings were stored individually at −20° C until tissue extraction and subsequent genetic analysis.

2.2. Haemosporidian detection

We extracted DNA from approximately 500 mg of muscle tissue of frozen wings using a DNeasy Blood and Tissue Extraction Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. In order to confirm the viability of each DNA extraction, a 695 base pair (bp) fragment of the avian mitochondrial DNA (mtDNA) cytochrome oxidase I (COI) gene was amplified following protocols described by Kerr et al. (2007). Each sample was considered viable if bands were visible when PCR product was visualized on 0.8% agarose gels stained with Gel Red Nucleic Acid Gel Stain (Biotium, Hayward, CA). Each extracted DNA sample that proved viable via our COI positive control ($n = 459$) was subsequently screened for the presence of *Haemoproteus, Plasmodium,* and *Leucocytozoon* parasites using a nested-PCR protocol described by Hellgren et al. (2004), which allows for simultaneous identification of infections from all three parasite genera. All samples were analyzed twice and visualized on 0.8% agarose gels as described previously. A 479 bp fragment of haemosporidian mtDNA cytochrome *b* gene was bi-directionally sequenced for all positive samples using identical primers from PCR. Positive PCR products were purified with ExoSapIT (USB Inc., Cleveland, OH) according to the manufacturer's protocol, and sequencing was conducted using Big Dye Terminator v3.1 mix (Applied Biosystems, Foster City, CA) and analyzed on an ABI 3730xl automated DNA sequencer (Applied Biosystems, Foster City, CA). Sequence data were cleaned up and edited using Sequencher 5.0.1 software (Gene Codes Corp., Ann Arbor, MI). Raw