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Avian malaria in a boreal resident species: long-term temporal variability, and increased prevalence in birds with avian keratin disorder

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ABSTRACT

The prevalence of vector-borne parasitic diseases is widely influenced by biological and ecological factors. Environmental conditions such as temperature and precipitation can have a marked effect on haemosporidian parasites (*Plasmodium* spp.) that cause malaria and those that cause other malaria-like diseases in birds. However, there have been few long-term studies monitoring haemosporidian infections in birds in northern latitudes, where weather conditions can be highly variable and the effects of climate change are becoming more pronounced. We used molecular methods to screen more than 2,000 blood samples collected from black-capped chickadees (*Poecile atricapillus*), a resident passerine bird. Samples were collected over a 10 year period, mostly during the non-breeding season, at seven sites in Alaska, USA. We tested for associations between *Plasmodium* prevalence and local environmental conditions including temperature, precipitation, site, year and season. We also evaluated the relationship between parasite prevalence and individual host factors of age, sex and presence or absence of avian keratin disorder. This disease, which causes accelerated keratin growth in the beak, provided a natural study system in which to test the interaction between disease state and malaria prevalence. Prevalence of *Plasmodium* infection varied by year, site, age and individual disease status but there was no support for an effect of sex or seasonal period. Significantly, birds with avian keratin disorder were 2.6 times more likely to be infected by *Plasmodium* than birds without the disorder. Interannual variation in the prevalence of *Plasmodium* infection at different sites was positively correlated with summer temperatures at the local but not statewide scale. Sequence analysis of the parasite cytochrome *b* gene revealed a single *Plasmodium* spp. lineage, P43. Our results demonstrate associations between prevalence of avian malaria and a variety of biological and ecological factors. These results also provide important baseline data that will be informative for predicting future changes in *Plasmodium* prevalence in the subarctic.

1. Introduction

Ecological factors can affect the diversity and distribution of vector-borne pathogens. In northern climates, where the transmission season may be limited, local environmental effects may significantly influence disease dynamics. Study of a resident northern passerine species provides an ideal system in which to examine the influence of environmental factors and host traits on parasite transmission and prevalence of infection.

Blood parasites of the order Haemosporidia, which infect amphibians, reptiles, birds and mammals and are transmitted by blood-sucking insect vectors, are one of the best-studied groups of parasites (Valkiūnas, 2005). Three genera of haemosporidians cause malaria and malaria-like diseases: *Plasmodium* (transmitted by mosquitoes, Culicidae), *Haemoproteus* (transmitted by biting midges, Ceratopogonidae), and *Leucocytozoon* (transmitted by black flies, Simuliidae). Asexual reproduction occurs in the vertebrate (intermediate host), while sexual reproduction occurs in the vector (definitive host). Avian malaria, caused by parasites of the genus *Plasmodium*, is responsible for the extinction and endangerment of numerous bird species (Beier and Stoskopf, 1980; van Riper et al., 1986; LaPointe et al., 2012). Morbidity is more likely in birds that have not evolved with *Plasmodium*; sublethal effects on host fitness such as mate selection, reproductive success and immune response are seen in wild bird populations that have long-standing associations with the parasite (LaPointe et al., 2012). Wild birds may retain mild chronic infections, potentially

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accumulating adverse side effects that impair genetic fitness or reduce their life span (Asghar et al., 2015).

Individual host characteristics may play an important role in exposure to vector-borne parasites. Adults commonly have higher prevalence than juveniles (Cosgrove et al., 2008; Atkinson and Samuel, 2010), a pattern which may be attributed to a longer period of exposure to vectors (Valkiūnas, 2005). The influence of sex on parasite prevalence is less clear, but some studies have reported a difference between males and females (Schall and Marghoob, 1995; Wood et al., 2007). In addition, individual health or disease status can also affect susceptibility to blood parasite infection (Knowles et al., 2011).

The occurrence of avian blood parasites can also be influenced by site characteristics, interannual or seasonal variation, temperature and precipitation (Lachish et al., 2011; Sehgal et al., 2011; Ramey et al., 2012; Gonzalez-Quevedo et al., 2014; Oakgrove et al., 2014; Krama et al., 2015). The relationship between climate and the prevalence of haemosporidians has been documented, with warmer temperatures facilitating the development of parasites (Freed et al., 2005; Garamszegi, 2011; Loiseau et al., 2012a, 2013; Zamora-Vilchis et al., 2012) as well as the abundance and development of vectors (LaPointe et al., 2012; Zamora-Vilchis et al., 2012). Multi-year studies including the effect of temporal, biotic and abiotic factors on haemosporidian infections have shown uneven prevalence across years (Fallon et al., 2004; Bensch et al., 2007; Clark and Clegg, 2015), highlighting the likely interactions of abiotic, vector and host-related factors on parasite prevalence.

Previous research has demonstrated that haemosporidians not only occur but are locally transmitted in Alaska, USA (Ramey et al., 2014, 2015), with Loiseau et al. (2012a) providing the first evidence of *Plasmodium* transmission in the state. Locally acquired infections of *Haemoproteus* and *Leucocytozoon* have been documented in the white-winged crossbill (*Loxia leucoptera*), a species that is a year-round resident of the boreal forest zone (Deviche et al., 2010). *Leucocytozoon* was found in both current and historic samples of goslings from tundra-nesting geese, indicating local transmission of the parasite for at least 20 years (Ramey et al., 2014). In the migratory Swainson's thrush (*Catharus ustulatus*), juveniles in Alaska were infected with *Leucocytozoon*, indicating that they had obtained the parasite locally (Dodge et al., 2012). A recent study across a broad latitudinal gradient in Alaska showed that the prevalence of four genera of haematozoa in 47 avian species varied with location, age, avian species, migratory status and, for *Leucocytoozoon*, co-infection by other parasites (Oakgrove et al., 2014). However, with the exception of Ramey et al. (2014), these studies were based on data collected during only 1 or 2 years, highlighting the need for analysis of a broad, long-term data set from a northern system to understand spatiotemporal patterns of infection at northern latitudes.

We capitalised on an extensive collection of blood samples gathered in Alaska during the course of an ongoing, long-term study of the black-capped chickadee (*Poecile atricapillus*), hereafter "chickadee." Chickadees form a monogamous pair bond and are strongly territorial during the breeding season before joining with neighbouring birds to forage in small flocks over their combined territories in the autumn and winter (Smith, 1991). Flocks have a dominance hierarchy, with an individual's rank affecting its behaviour and chances for survival (Smith, 1991). Age, sex, size and social dominance all play a role in this hierarchy and females may avoid foraging in microhabitats used by dominant males (Desrochers, 1989). The dynamics between the age and sex groups make for possible differences in the microhabitats to which each is exposed, potentially allowing for a variance in parasite prevalence within the species.

Recently, chickadees and other species in Alaska and the Pacific Northwest have been affected by a condition called avian keratin disorder (Handel et al., 2010). This disease, which was the primary focus of the original study and whose aetiology is still unknown, causes rapid growth of the outer keratinized layer of the bill and may also affect the skin, legs, feet, claws and feathers (Handel et al., 2010; Van Hemert and Handel, 2010; D'Alba et al., 2011; Van Hemert et al., 2013). The resulting beak deformity reduces the bird's ability to preen and some affected individuals have been found heavily infested with parasitic feather mites (Handel et al., 2010). Birds with this disorder alter their dietary habits and behaviour, and their reproductive fitness, health and survival are also negatively affected (Handel et al., 2010; Van Hemert et al., 2012).

Here we investigate environmental and host factors associated with the prevalence of infection in a resident subarctic passerine bird by the haemosporidian parasites *Plasmodium* and *Haemoproteus*. We describe the dynamics of haemosporidian prevalence in populations of wild-caught chickadees over a 10 year period, primarily during winter, at several sites in Alaska. We aimed to identify possible relationships between parasite prevalence and variables associated with the local environment including summer climate, season, location and year. We also sought to evaluate the relationship between parasite infection and characteristics of the individual host, including age, sex and disease status. We predicted that the prevalence of haemosporidian infection in resident birds at different sites during winter would be positively correlated with climatic variables (temperature and precipitation) at those sites during the previous summer, when vectors are active and parasite transmission may occur. We also predicted a seasonal decline each winter as individuals possibly cleared infections acquired during the previous summer. We predicted that the prevalence of parasite infection would be higher among adult than juvenile birds due to differences in exposure to vectors during summer and that the prevalence might differ between sexes if birds used different microhabitats due to the birds' dominance hierarchy (Smith, 1991). Finally, we hypothesised that birds affected by avian keratin disorder would have a higher prevalence of haemosporidian parasites than unaffected birds, due to possible health effects that the disorder has on the individual.

2. Materials and methods

2.1. Sample collection

We collected 2070 blood samples from chickadees at seven sites in southern central and interior Alaska between the years 2000 and 2011 (Fig. 1), primarily during the non-breeding season, as part of a long-term capture–recapture study of avian keratin disorder in this species (Handel et al., 2010). Non-breeding chickadees were captured systematically every 2 months between September and April (hereafter 'winter') using modified funnel traps and mist nets at three 10 ha sites in wooded parklands surrounded by residential areas in the greater Anchorage, USA, area (Campbell Creek Science Center, Eagle River Nature Center, Mirror Lake Middle School in Chugiak). Additional non-breeding birds were captured during various years at two rural residences in southern and western Anchorage, on the wooded campus of Matanuska-Susitna College, on the wooded campus of the University of Alaska Fairbanks, and on the Kenai National Wildlife Refuge (Table 1, Fig. 1). Study areas ranged in elevation from 56 to 155 m above sea level and all were in mature mixed forests dominated by white spruce (*Picea glauca*) and paper birch (*Betula papyrifera*) interspersed with lowland black spruce (*Picea mariana*) bogs. A few blood samples collected during a nest-box study in the Anchorage area during the summers of