Parasite Prevalence in Dark-eyed Juncos, *Junco hyemalis,* Breeding at Different Elevations

HEATHER BEARS

Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia V6T 1Z4 Canada; e-mail: bears@zoology.ubc.ca

Present address: Centre for Applied Conservation Research, 2424 Main Mall, Forest Sciences Building, University of British Columbia, Vancouver, British Columbia V6T 1Z4 Canada

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During the summer of 2001, Dark-eyed Juncos (*Junco hyemalis*) were captured within the lowest (1000 m above sea level) and highest (2000 m asl) elevation extremes of their breeding range in Jasper National Park, Alberta. Blood samples were taken to identify parasite genera, and to test for differences in parasite prevalence among elevations. The most common parasites at either elevation were *Haemoproteus* spp., *Leucocytozoon* spp., and *Trypanosoma* spp. A significantly higher proportion of low- compared to high-elevation birds was infected by at least one of these, supporting the prediction that high-elevation habitats may be refuges from parasites.

Key Words: Dark-eyed Juncos, *Junco hyemalis*, blood parasites, mountains, elevation, Alberta.

Birds breeding at high elevations often experience a delayed date of reproductive onset, exposure to extreme weather, and scarce or sparsely distributed resources (Hamman et al. 1989; Landmann and Winding 1993; Kollinsky and Landmann 1996; Widmer 1999; Bears et al. 2003). Dark-eyed Juncos (*Junco hyemalis*) show a significant reduction in seasonal reproductive output with increasing breeding elevation from the montane valley (1000 m asl) to the subalpine-alpine treeline (2000 m asl) in Jasper National Park, Alberta (Bears 2002, Bears et. al. 2003). Yet, high elevation habitats are not occupied by less competitive age or size classes of juncos, or by later arriving individuals (Bears 2002), all of which are traits that render juncos less successful competitors for territory (Cristol et al. 1990; Grasso et al. 1996). Further, inter-annual return of birds to their site of capture is high, and roughly equivalent among elevations (Bears 2002). Hence, it does not appear that juncos are "forced" to breed at high elevations due to intraspecific competitive exclusion from lower-elevation habitat. This led me to explore the idea that high-elevation juncos are compensated for decreased seasonal reproductive output over their lifetimes by some unrecognized benefits of breeding at high elevations. In other mountain ranges, studies have shown that the blood parasites of birds, and the insect vectors that carry them, tend to be low at upper breeding elevations (Stabler et al. 1974; Braun et al. 1993). Thus, as part of a series of investigations into the benefits of high-elevation breeding, I tested whether the juncos have fewer parasites within higher- versus lower-elevation segments of their breeding range in Jasper.

Methods

Juncos were captured and monitored at eight 50 – 70 ha study sites in Jasper National Park (52º53'N, 118º3'W), Alberta, from 1 May to 20 August in 2000, and from 15 April to 20 August in 2001. Four sites were located near the lowest elevation within the Park (1000 – 1020 m asl), separated by 5-10 km. Four sites were at the highest elevation at which juncos breed within the park (1950-2100 m asl), and were separated by > 18 km. All high-elevation sites had south or southest aspects. Morphological measurements taken from juncos in these sites (H. Bears, unpublished data) most closely match the Oregon subspecies, *Junco hyemalis oregonus* (Miller 1941), but some appear to be intergrades between *Junco hyemalis oregonus* and *Junco hyemalis hyemalis*, which produces *Junco hyemalis cismontanus* (Miller 1941). Subspecies designations of Dark-eyed Juncos are a capricious topic based on phenetic rather than genetic data, and they are a taxonomic nightmare in the Canadian Rockies where multiple subspecies meet and hybridize. Therefore, we refer to the birds analyzed here simply as *Junco hyemalis.*

Birds were captured using Japanese mist nets with painted model male juncos as decoys. Juncos were lured towards the net by playing the taped song of a conspecific. Birds were given a numbered Canadian Wildlife Service leg band and colour bands that conveyed sex, age, and site information when caught. Blood samples were taken from each bird caught between 1 May and 20 August in 2001 only by puncturing the alar vein with a needle and collecting ca. 40 µl of blood in a heparinized microhematocrit tube.

Blood was blown out of microhematocrit tubes into centrifuge tubes and kept on wet ice. Because blood parasites may follow a diurnal periodicity (Gore et al. 1982), the birds selected for parasite identification were caught at approximately the same time each day (7:00-12:00 hrs). Within 10 hours of collection, 5 µl of blood were used to produce three microscope slides per bird, using the methods of Harrison and Harrison (1986) and Bennett (1970). The slides were fixed in 100% methanol immediately. In the laboratory, blood smears were stained with Geimsa stain for 30 minutes, and rinsed with distilled water followed by acetone under a fumehood (Deviche et al. 2001). Smears were examined under 400 × 10 magnification, and 50 fields of view per slide were classified as negative or positive for various parasites. Parasites were identified to the genus level by examination under high power (1000 × magnification) with oil immersion, using various keys (Pierce 1981; Bennett and Pierce 1988; Burrey-Caines and Bennett 1992; Bennett et al. 1994), and by comparing with photos of blood parasites taken from juncos captured in Alaska (supplied by Pierre Deviche, Arizona State University, personal communication). Parasite species were difficult to ascertain with absolute certainty, but the genus level could be resolved without ambiguity. Therefore, we used the genus level when comparing parasite prevalence among elevations.

At each site, one bird was caught between the 1st and 5th of each month (May-August), and another between the 11th and 15th of each month, in order to represent sites and time periods within the breeding season equivalently. No females were captured using playback and mist-netting methods, and so analyses here deal solely with males. The statistical significance level for all tests conducted was set at $\alpha = 0.05$. All tests were performed using SPSS 10. All techniques used were approved by the animal care committee of the University of British Columbia (A0-0046), Parks Canada (2000-008), and Environment Canada (Banding: 10429 AJ; Collection: BC SCI 2000/067).

Results

Thirty-two high- and 32 low-elevation adult male birds caught across all eight sites were used in these analyses. In addition, 12 fledglings were caught at high elevations (in August) and 12 were caught at low elevations (in August). Three parasite genera were found in the blood of juncos: *Haemoproteus* (Family Plasmodiidae), *Leucocytozoon* (Family Plasmodiidae) and *Trypanosoma* (Family Trypanosomatidae). All data are summarized in Table 1. The most prevalent blood parasite at both elevations was *Haemoproteus*, followed by *Leucocytozoon,* and *Trypanosoma*. A significantly higher proportion of low-elevation birds (66%, 21/32) compared to high-elevation birds (28%, 9/32) were infected by at least one of these parasites (P = 0.002, Fisher's Exact Test). A higher percentage of low-elevation birds (34%, 11/32) compared to high-elevation birds (19%, 6/32) were infected with *Haemoproteus* spp., but the difference was not significant (P = 0.08, Fisher's Exact Test). Similarly, more low-elevation birds (25%, 8/32) versus high-elevation birds (9%, 3/32) were infected with *Leucocytozoon* spp., with a near significant difference (P = 0.07, Fisher's Exact Test). Of these individuals, 42% (8/19) of low-elevation and 44% (4/9) of high-elevation birds had both *Haemoproteus* spp. and *Leucocytozoon* spp. present in their blood. Two cases of infection with *Trypanosoma* spp. were found in low-elevation birds, and none at high elevations. Thirty-four percent (11/32) of low-elevation and 72% (23/32) of high-elevation birds were not infected with any parasites. There was no relationship between date of capture and infection at low elevations (P = 0.23, r = 0.33; Pearson's r). However, at high elevations, a low proportion of individuals (12.5%, 2/16) caught between 1 May and 15 June were infected with *Leucocytozoon* spp. and *Haemoproteus* spp., but between 15 June and 30 August, a significantly higher proportion were infected (7/16, 44%; P = 0.05, Fisher's Exact Test). In samples from low elevations not used in this analysis (taken from birds nearby, but not within our study areas) two cases

TABLE 1. Summary of data on presence and absence of blood parasites in Dark-eyed Juncos from low (~1000 m asl) and high (~2000 m asl) elevations.

Adults (ASY)	*Infection/Parasite*	*Low (N = 32)*	*High (N = 32)*
	Infected	21	9
	Trypanosoma present	2	0
	Leucocytozoon present	8	3
	Haemoproteus present	11	6
	Both *Haemoproteus* and *Leucocytozoon* present	8 of 19 individuals	4 of 9 individuals
	no parasites	11	23
Fledglings	*Parasite*	*Low (N = 12)*	*High (N = 12)*
	Leucocytozoon *present*	2	1

of *Plasmodium* spp. (family Plasmodiae) were noted. More fledglings were infected with *Leucocytozoon* spp. at low elevations as compared to at high elevations (17%, 2/12, vs 8.3%, 1/12), but the difference was not significant (P = 0.39, Fisher's Exact Test). No other blood parasites were observed in fledglings.

Discussion

High-elevation adult males and fledglings had lower incidences of blood parasites as compared to low-elevation adult males and fledglings. If relief from parasites enables adults to survive longer and breed for more years, then this could aid in equalizing the lifetime reproductive success of high- and low-elevation birds. Blood parasites can decrease survivorship by directly increasing susceptibility to predation (Vaughn and Coble 1975). In addition, there may be a tradeoff between reproductive effort and the efficiency of the immune response. For instance, parasite loads may increase during the reproductive period, as they did here at high elevations, when breeding adults spend considerable time provisioning their young and in nest and territorial defence, or when breeding effort is increased experimentally (Ots and Horak 1996; Norris et al. 1994; Weatherhead and Bennett 1991, 1992; Rintmaki et al. 1999). A simpler reason for the differential susceptibility observed here might also be due to a difference in the timing of emergence of the insect vectors carrying the parasites.

The two most common parasite genera in this study, *Haemoproteus* and *Leucocytozoon*, are protozoon parasites of birds. *Haemoproteus* spp. are primarily transmitted by insects in the dipteran family Ceratopogonidae (no-see-ums, sandflies) or Hippoboscidae (louse flies), whereas *Leucocytozoon* spp. are primarily transmitted by simuliids (e.g., black flies) (Greiner and Ritchie et al. 1994; Rosskopf and Woerpel 1996; Rintmaki et al. 1999). *Trypanosoma* (likely *avium*), which was rare in our study, is also transmitted by members of the family Simuliidae. The principal effects of *Leucocytozoon* infections are intravascular haemolytic anemia, weight loss, and sometimes death, whereas *Haemoproteus* and *Trypanosoma* in birds are generally less pathogenic (Greiner and Ritchie 1994). Finally, two low-elevation samples (not analyzed as part of the sub-samples selected) contained *Plasmodium* spp., which is transmitted by mosquitos (family Culicidae). Almost all of these insect vectors were noted to have emerged later at high elevations, perhaps preventing exposure of birds to the parasites for much of the season. High-elevation birds were also at lower densities (Bears 2002), and therefore transmission between birds may have been lower.

Results suggest that high-elevation habitats may be of conservation importance in limiting the spread of blood parasites in birds. The Canadian Rockies may play a particularly important role in limiting the rate at which avian diseases travel east and west across the Rocky Mountains. Further work, including comparisons of parasite levels in females, and in other species that breed over wide elevation ranges, is required in order to assess the generality of this parasite refugium hypothesis in the Rocky Mountains of Canada. Mechanisms by which high-elevation birds are protected from parasites should also be explored.

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