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Effects of Time of Sampling on Oocyst Detection and Effects of Age and Experimentally Elevated Testosterone on Prevalence of Coccidia in Male Dark-eyed Juncos

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The hormone testosterone (T) has been linked to enhanced expression of secondary sexual traits and acquisition of mates (Ligon et al. 1990, Alatalo et al. 1996, Raouf et al. 1997). However, high plasma levels of testosterone also have been suggested to suppress immune function (Folstad and Karter 1992, Zuk et al. 1995, Hillgarth and Wingfield 1997; but see Ros et al. 1997, Hasselquist et al. 1999). Thus, an obvious question is whether testosterone enhances reproductive success while simultaneously affecting susceptibility to disease.

In the Dark-eyed Junco (*Junco hyemalis*), experimental maintenance of plasma testosterone levels at their natural early season peak for the entire breeding season causes males to sing more frequently, and testosterone-treated males (T-males) in captivity are more attractive to females than are control males (C-males; Enstrom et al. 1997, Hill et al. 1999). However, T-males also have elevated plasma levels of the steroid hormone corticosterone (Ketterson et al. 1991, Klukowski et al. 1997) and show a greater response to handling stress than do C-males (Schoech et al. 1999), which can translate into suppressed immune function (Sapolsky 1992, Besedovsky and del Rey 1996, J. Casto pers. comm.). To investigate possible effects of elevated levels of plasma testosterone on the junco's susceptibility to disease, we asked whether free-living males treated with testosterone would be more likely than controls to be infected by coccidia (*Isospora* spp.), a protozoan gut parasite belonging to the *Eimeria* complex. We also asked whether yearling juncos were more likely than older adults to be infected and whether our ability to detect infection was affected by the time of day when fecal samples were collected.

Methods.—This research was conducted at the University of Virginia's Mountain Lake Biological Station (37°22′N, 80°32′W; see Chandler et al. [1994] for description of study area). We captured male juncos in mist nets and Potter traps during the early spring of 1995 and 1996 and classified them as second year (younger) and after second year (older) based on plumage characteristics and iris coloration (Pyle et al. 1987, Mulvihill 1993). Males were randomly assigned a treatment and implanted with either control or testosterone-filled implants that consisted of two 10-mm lengths of silastic tubing placed subcutaneously above the thigh and under the wing (see Ketterson et al. 1992). T-implanted males maintained plasma testosterone levels that corresponded to the natural peak found in untreated males during territory establishment and pair formation (Chandler et al. 1997) and were higher than those of C-males (Ketterson and Nolan 1992).

Males captured in late summer (30 T-males, 23 C-males) were taken into the laboratory and placed in individual holding cages (22 × 28 × 30 cm) lined with fresh wax paper. Food and water were available *ad libitum*. In both years, samples produced between 1500 and 0500 (EST) were considered "night" samples, and those produced between 0500 and 1500 were considered "day" samples. Only night samples were used in analyses of the effects of testosterone and age on prevalence of infection, but samples collected at both times were used to determine whether time of day affects whether feces contained oocysts. In addition, we sampled feces produced by 14 individuals during both day and night in 1996. These males were brought into the laboratory after 1500 and held overnight for feces collection. Their cage linings were changed the following morning at 0500, and the birds were held until 1200.

Feces and residual food were separated by hand and the food discarded. Feces were placed in petri dishes containing approximately 7 mL of a 2.5% potassium dichromate solution for seven days, which is adequate time for oocyst sporulation to occur (D. W. Duszynski pers. comm.). After sporulation, samples were transferred from petri dishes to vials for storage. We followed the methods of Duszynski and Wilber (1997) for separating oocysts from fecal material and identifying them. Birds were classified as infected (positive) if we detected one or more oocysts when examining samples under a microscope. Samples were scanned only once if positive and twice if the first scan was negative; the latter samples were considered to be positive if one or more oocysts were detected.

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Coccidia typically are transmitted when a potential host ingests an infective propagule; the likely mode of transmission in juncos is via ground feeding. Although little is known about the coccidia that infect juncos, oocysts discharged by juncos belonged to the genus *Isospora* based on oocyst morphology (four sporozoites within each of two sporocysts). In addition, when we measured the length and width of the oocysts, the frequency distribution for each measurement was unimodal, which is consistent with infection by a single species.

Statistical analyses were performed using SYSTAT 5.0. We first performed pairwise *G*-tests to determine if detection of oocysts was dependent on the time that samples were collected. Because prevalence depended on when samples were collected (see Results), subsequent analyses were based on night samples only. We used a loglinear model to determine whether prevalence of coccidia was dependent on hormone treatment or age, as well as to determine if hormone treatment and age influenced prevalence independently.

Results.—Coccidial oocysts occurred more frequently in feces produced at night in both 1995 (night, 23 of 31; day, 3 of 15; $G = 12.57$, df = 1, $P < 0.001$) and 1996 (night, 19 of 22; day, 6 of 12; $G = 5.14$, df = 1, $P < 0.01$). For the 14 individuals sampled in both day and night, oocysts occurred more often in feces produced at night (day, 2 of 14; night, 11 of 14; $G = 12.64$, df = 1, $P < 0.001$). Therefore, subsequent analyses were based only upon samples produced at night.

Because the prevalence of coccidia did not differ significantly between years ($G = 1.20$, df = 1, $P = 0.27$), we combined data to assess the overall effect of hormone treatment, age, and the interaction of hormone and age on prevalence of coccidia in male juncos. Neither hormone treatment ($G = 0.00$, df = 1, $P = 0.97$) nor age ($G = 1.17$, df = 1, $P = 0.28$) influenced prevalence of coccidia (Fig. 1). In addition, the loglinear model revealed no interaction between hormone treatment and age ($G = 1.09$, df = 1, $P = 0.30$).

Discussion.—Laboratory experiments on House Sparrows (*Passer domesticus*) indicate that *Isospora* coccidia are shed on a circadian rhythm, with the highest numbers of oocysts being shed between 1600 and 0100 (Boughton 1988). Our field experiments on Dark-eyed Juncos strongly agree with these results. In random samples from both years, coccidial oocysts were more likely to be found in samples collected at night than during the day. Further, in 14 birds sampled during both day and night, we were more likely to detect oocysts in feces produced at night. Therefore, we recommend that future studies of coccidia focus on samples collected at night.

In juncos, treatment with testosterone results in an increase in plasma levels of testosterone (Ketterson and Nolan 1992) and corticosterone (Ketterson et al. 1991, Klukowski et al. 1997). Although increased plasma testosterone and corticosterone are correlated with suppressed immune function in salmonid fish (Slater and Schreck 1993) and mammals (Hillgarth and Wingfield 1997), the effect of testosterone on avian immune function is not clear (Marsh 1992). Some studies suggest that testosterone is immunosuppressive in birds (Saino et al. 1995, Zuk et al. 1995), whereas others provide no support for such a relationship (Ros et al. 1997, Hasselquist et al. 1999). Treatment with T suppresses cell-mediated immunity and humoral immunity in juncos (J. Casto pers. comm.), but we found no evidence that experimentally elevated T produces a detectable effect on the prevalence of coccidia. Background prevalence of coccidia was high in our population, however, and our sample sizes were small. In addition, we had no measure of the intensity of infection. Therefore, we conclude that although we found no effect, another study of the effect of T on susceptibility to coccidia, especially one employing larger sample sizes and a measure of intensity of infection, might be warranted.

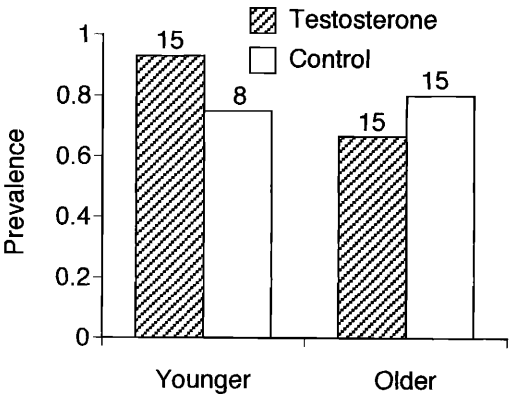

FIG. 1. Prevalence of coccidia by age in testosterone-treated and control male Dark-eyed Juncos at Mountain Lake Biological Station, Virginia. Data are prevalence estimates pooled between the 1995 and 1996 breeding seasons (sample sizes shown above bars).

Laboratory studies of domestic chickens experimentally infected with coccidia (*Eimeria* spp.) revealed lower prevalence in adult birds, which was interpreted as indicating that older chickens had more time to build partial immunity to infection (Levine 1988). Similarly, younger male juncos were more likely to be infected than were older birds, but the effect of age was not significant (Fig. 1).

In summary, suppressed immune function has been suggested as a cost of elevated testosterone levels. Therefore, we tested whether male Dark-eyed Juncos with experimentally elevated testosterone would be more likely to harbor coccidia (*Isospora*

spp.), which are protozoan parasites that may cause the disease coccidiosis. The likelihood that an individual would shed coccidial oocysts depended on the time of day when feces were produced, supporting earlier laboratory studies by Boughton (1988). However, we found no evidence that treatment with testosterone or an individual's age affected the likelihood that it would harbor coccidia, and thus we found no evidence to support a tradeoff between reproduction and survival.

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Identification of the Extinct Hawaiian Eagle (*Haliaeetus*) by mtDNA Sequence Analysis

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Among the many bones of extinct birds discovered in the first extensive deposits studied in the Hawaiian Islands were a few from Molokai and Oahu that clearly belonged to a large sea-eagle of the genus *Haliaeetus*, as shown by their large size and the fusion of the phalanges of the inner toe (Olson 1982, Olson and James 1982). Even with the discovery of a nearly complete skeleton of this eagle on Maui, its specific identification could not be entirely resolved. Size and other characters eliminated most species of the genus, but no qualitative osteological characters could be discerned between the Hawaiian fossils and available skeletons of the Old World White-tailed Eagle (*H. albicilla*) and the New World Bald Eagle (*H. leucocephalus*; Olson and James 1991). Unable to make a morphological resolution, Olson and James (1991:62) were constrained to list this interesting specimen as "*Haliaeetus* sp., aff. *H. leucocephalus*/*H. albicilla*." To identify the Hawaiian eagle more satisfactorily, we turned to "ancient" DNA methods to obtain mitochondrial DNA (mtDNA) sequences from its bones.

The bones used in ancient DNA analysis were obtained from a nearly complete skeleton found on 4 April 1988 in a good state of preservation in Puu Makua Cave, a lava tube at 1,463 m elevation on the south slope of Mt. Haleakala, Maui (Olson and James 1991). The eagle had died in a cave that served as a natural trap for flightless species such as moa-nalos, rails, and ibises. The only entrance to the cave is a skylight of approximately 5 m diameter above a vertical drop of approximately 22 m. The eagle may have flown into the cave to scavenge flightless birds that had fallen in beforehand. The eagle presumably became trapped because the entrance was too small for such a large bird to be able to fly back out.

Previous analyses showed that the skeleton contained 4.1% nitrogen and amino acids in proportions only slightly different from those of modern cow bone (T. Stafford, Jr. pers. comm.). An AMS radiocarbon date on the skeleton gave an age of 3,300 ± 60 years (two-sigma calibrated range 3,389 to 3,689 years; see James 1995). Because the nitrogen and amino acid content suggested that organic compounds

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