

Year-Class Differences in the Reproductive System, Plasma Prolactin and Corticosterone Concentrations, and Onset of Prebasic Molt in Male Dark-Eyed Juncos (*Junco hyemalis*) during the Breeding Period

Pierre Deviche,^{*,1} John C. Wingfield,[†] and Peter J. Sharp[‡]

^{*}Department of Biology, Arizona State University, Tempe, Arizona 85287-1501; [†]Department of Zoology, University of Washington, Seattle, Washington 98195; and [‡]Roslin Institute (Edinburgh), Roslin, Midlothian EH25 9PS, United Kingdom

Accepted February 14, 2000

Year-class differences in reproductive function were investigated in a free-living population of adult male Dark-eyed Juncos, *Junco hyemalis*, breeding in interior Alaska. Second-year males (SY, entering their first breeding season) were compared with after-second-year males (ASY, entering at least their second breeding season). We measured body mass, size of the cloacal protuberance (CP), testis mass, onset of prebasic molt, and concentrations of plasma luteinizing hormone (LH), testosterone (T), corticosterone (CORT), and prolactin (PRL) throughout the reproductive season (April to mid-July). There were no differences in SY and ASY body weights but SY males had smaller CPs and testis masses than ASY males during gonadal recrudescence and at the end of the breeding season. Plasma LH was elevated from April until mid-June and then decreased in the same way in both year classes. In contrast, plasma T was high from April until mid-May and was lower in SY than in ASY juncos shortly after they arrived on their breeding grounds at the end of April, but not at other times. In July, SY males started to molt earlier, suggesting that they became photorefractory earlier than ASY males. Plasma PRL increased progressively in both year classes between April and early June and decreased in early July. At this time, plasma PRL

decreased earlier in SY than in ASY males. Plasma CORT changed seasonally, but did not differ between SY and ASY juncos. Thus, year-class differences in CP sizes and testis mass apparently did not result from SY males secreting less LH or more PRL or CORT than ASY males. It is suggested that differences in reproductive condition in SY and ASY juncos are mediated by interactions with conspecific birds and do not result from an intrinsic effect of age. © 2000 Academic Press

Key Words: reproduction; luteinizing hormone; LH; photoperiodism; photorefractoriness; seasonal breeding; gonads; testosterone; testis; androgen.

INTRODUCTION

Most species of birds breeding at middle and high latitudes use photoperiod as the main environmental factor for timing the onset and the end of their annual reproductive period. In these species exposure to day-length exceeding approximately 12 h (long days, LD), as is normally the case in spring, promotes the release of gonadotropin-releasing hormone. Continued exposure to LD eventually leads to a centrally mediated desensitization of the hypothalamo-pituitary-gonadal axis to the stimulating effects of long days and

¹ To whom correspondence and reprint requests should be addressed. Fax: (480) 965-2519. E-mail: deviche@asu.edu.

birds become photorefractory (for reviews, see Nicholls *et al.*, 1988 and Juss, 1993). The transition from photosensitive to photorefractory condition takes place in summer, when photoperiod is still well in excess of the vernal threshold for stimulation (Wingfield *et al.*, 1980; Dawson, 1983; Stokkan *et al.*, 1986; Parry *et al.*, 1997). In most species, photorefractoriness is signaled not only by rapid gonadal involution but also by onset of prebasic (or postnuptial) molt (Morton *et al.*, 1969; Dawson, 1997; Dawson and Sharp, 1998).

In addition to stimulating the hypothalamo–pituitary–gonadal axis, exposure of photosensitive birds to LD induces the release of pituitary prolactin (PRL; Wingfield and Goldsmith, 1990). Photoinduced plasma PRL levels are generally highest at the onset of reproductive photorefractoriness and then decline gradually (European Starling, *Sturnus vulgaris*: Dawson and Goldsmith, 1983; Dawson, 1997; Dawson and Sharp, 1998). Although PRL administration inhibits the reproductive system (Buntin and Tesch, 1985; Janik and Buntin, 1985), this hormone is not considered responsible for induction of photorefractoriness (Juss, 1993; Sharp *et al.*, 1998). However, it probably contributes to the rapid inactivation of the pituitary–gonadal axis that occurs at the onset of photorefractoriness and it may stimulate prebasic molt (Dawson and Sharp, 1998).

The activity of the reproductive system and plasma concentrations of pituitary–gonadal hormones can be modulated by numerous factors in addition to photoperiod. These factors include weather conditions (Wingfield *et al.*, 1983; Wingfield, 1985a), especially temperature (Engels and Jenner, 1956), food availability (Ligon, 1974), social interactions with conspecific young and adults (Schwab and Lott, 1969; Delville *et al.*, 1984; Dufty and Wingfield, 1986; Wingfield and Wada, 1989; Ketterson *et al.*, 1990; Silverin and Westin, 1995; for review, see Wingfield *et al.*, 1994), and breeding effort (Wingfield and Farner, 1979; Hegner and Wingfield, 1986; Ball and Wingfield, 1987). A small number of studies have also described effects of year-class on the reproductive system activity of adult birds. Specifically, plasma testosterone (T) and follicle stimulating hormone (FSH) concentrations are lower during the breeding season in young than in older adult male Dark-eyed Juncos (*Junco hyemalis*; Ketterson and Nolan, 1992) and Great Tits (*Parus major*; Silverin *et al.*, 1997), respectively. Captive yearling male Northern Pintails (*Anas acuta*) also have lower

plasma T levels than older males (Sorenson *et al.*, 1997). Similarly, free-living yearling Mountain White-crowned Sparrows (*Zonotrichia leucophrys oriantha*) have lower plasma T levels and smaller testes and cloacal protuberances (CP, an androgen-dependent male secondary character; Deviche, 1995; Schwabl and Farner, 1989) than older males during the reproductive period (Morton *et al.*, 1990). The two age classes of sparrows, however, have similar plasma luteinizing hormone (LH) concentrations and are equally successful in rearing young. Other investigations have uncovered effects of age on reproductive success (see review by Sæther, 1990), but the underlying bases of these effects remain poorly understood. Specifically, little information is available on whether the above differences are intrinsic to age or result from breeding experience.

The present investigation compares aspects of the reproductive morphology and endocrine physiology in free-living second-year (SY, birds hatched the previous year and entering their first breeding season) and older (after-second-year) (ASY, birds having undergone at least one photoinduced gonadal cycle) male Dark-eyed Juncos. Based on previous research on this species (Ketterson and Nolan, 1992) and on White-crowned Sparrows (Morton *et al.*, 1990), we predicted that SY males would have seasonally lower plasma T levels and smaller testis and CP sizes than ASY males, but similar plasma LH concentrations. In addition to measuring parameters of reproductive function, we determined plasma concentrations of corticosterone (CORT) and PRL, two hormones that inhibit the reproductive system (Wilson and Follett, 1975; Deviche, 1983) and may, therefore, play a role in the control of year-class differences in gonadal activity. We compared the timing of prebasic molt between the two age groups and examined whether this timing relates to differences in circulating T and PRL levels. Prebasic molt is inhibited by T (Nolan *et al.*, 1992; Deviche, 1995) and may be promoted by PRL (Dawson and Sharp, 1998).

MATERIALS AND METHODS

Subjects

A total of 257 adult male Dark-eyed Juncos were caught using either Japanese mist nets (93%) or seed-

baited Potter traps (7%) in the vicinity of Fairbanks, Alaska (65°N; 148°W) between end of April and July 1997. Based on 10 consecutive years of study (Deviche, personal observations), it appears that the time course of breeding events varies little from one year to another, especially at the beginning of the reproductive season. Birds belonging to this population generally arrive on breeding grounds at the end of April and early May. During this month, pairs are formed, nests built, and first-clutch eggs laid. Many young hatch at the end of May and early June. Young leave the nest when 11–12 days old (Ketterson *et al.*, 1992), at which time they continue to be fed by their parents for about 10 days before becoming independent. Pairs that lose their clutch will attempt to renest until approximately early July.

Mist-netted birds were attracted by playing taped conspecific songs. Most birds were caught within 5–10 min of the beginning of song playback and there was no evidence of either age group (see below) being differently responsive to playbacks than the other. Immediately after capture, blood (approximately 200 μ l) was collected from a brachial vein into heparinized microcapillary glass tubes. Blood samples were placed on ice until they were centrifuged (3000 rpm at 4° for 10 min) in the laboratory, generally within 5 h of collection. Plasma was collected and stored at –20° until assayed for T, CORT, PRL, and LH (see below). After blood collection, birds were weighed to the nearest 0.1 g and weights were corrected for the amount of blood removed. Wing chords and CP widths were measured to the nearest 1 and 0.1 mm, respectively. We also examined whether birds were undergoing prebasic primary feather molt. Juncos as well as most other small passerine birds undergo a complete (pre-basic) molt at the end of the breeding season. This molt starts with the loss and replacement of proximal primary wing feathers (Pyle, 1997). Whenever possible, males were categorized as SY or ASY based on plumage characteristics (Pyle, 1997). Five males that could not be assigned to either age category were excluded. Males received a U.S. Fish and Wildlife numbered aluminum leg band (Permit No. 22640) and in some cases a unique combination of plastic colored leg bands and they were released at the capture site. All required State and Federal Scientific/Educational collecting permits were obtained prior to the studies.

Insufficient volumes of plasma were available to assay samples collected in 1997 for PRL. Concentrations of this hormone were, therefore, measured in samples ($n = 217$) collected in 1996 under the same conditions and in the same area as in 1997.

To determine seasonal changes in testicular masses, we combined data obtained for 182 adult males of known age (SY or ASY) that were sacrificed between April and July, 1991–1997. The annual number of sacrificed males ranged from 2 (1991) to 46 (1995). Males were sacrificed on the same day as they were captured, either by decapitation or by *in vivo* intracardiac perfusion under complete anesthesia. Shortly after sacrifice, both testes were excised and weighed to the nearest mg. Testes that could not be weighed immediately after sacrifice were placed in 0.9% NaCl solution until weighing to prevent dehydration. The University of Alaska Institutional Animal Care and Use Committee approved all experimental protocols.

Hormone Assays

Testosterone, corticosterone, and luteinizing hormone. Total plasma T concentrations were measured using a commercial solid-phase radioimmunoassay (RIA) system (Diagnostic Products Co., Los Angeles, CA). Briefly, plasma (25 μ l) was added to T antibody-precoated plastic tubes. ¹²⁵I-labeled T solution (1 ml; approximately 44,000 dpm) was added to all the tubes that were then incubated in a water bath at 37° for 3 h. The content of the tubes was decanted and the antibody-bound radioactivity was measured in a γ counter. Plasma T concentrations were calculated by reference to a standard curve generated by incubating human plasma samples containing known T concentrations (0.1 to 16 ng/ml) under the same conditions as the junco plasma samples. The antibody used in the assay has low (<5%) cross-reactivity with CORT, estradiol, progesterone, and 5 α -dihydrotestosterone. A junco plasma dilution curve was parallel to the standard curve. T was undetectable in samples obtained from captive males that either were castrated or were chronically exposed to artificially short photoperiod, as well as from most field-sampled adult breeding females (Deviche and Gulledge, 2000).

In small passerine birds, plasma CORT concentrations increase rapidly following exposure to adverse conditions (Schwabl *et al.*, 1991; Astheimer *et al.*, 1994;

Wingfield *et al.*, 1994; Romero *et al.*, 1998a,b). Generally, this increase does not occur until at least 3 min after capture and handling. We measured plasma CORT levels as previously described (10–20 μ l plasma/tube; Wingfield *et al.*, 1992) and only in samples obtained from mist-netted males for which blood collection was completed within 3 min of capture. Results, therefore, represent baseline circulating hormone concentrations.

We measured plasma LH concentrations (10–20 μ l plasma/tube) using a double-antibody precipitation RIA as described by Follett *et al.* (1972) and modified by Follett *et al.* (1975). This assay uses purified chicken LH as the standard and detects the hormone in various passerine species (Wingfield *et al.*, 1982; Wingfield, 1985b; Wingfield and Wada, 1989; Saldanha *et al.*, 1994).

Prolactin. We determined plasma PRL levels (10 μ l plasma/tube) using a recombinant-derived European Starling PRL RIA (Bentley *et al.*, 1997). Recombinant-derived European Starling PRL was used to produce 125 I-labeled tracer and assay standards. A dilution curve generated by diluting an adult junco plasma pool was parallel to the standard curve. Plasma PRL was much higher in female juncos that were incubating or feeding young than in pre- or postbreeding females (Deviche and Sharp, unpublished results).

For each hormone, all samples were assayed in duplicate and in a single series. The assay sensitivities were 5 (T), 7.8 (CORT), 10 (LH), and 91 (PRL) pg hormone detectable/assay tube. Intraassay coefficients of variation averaged 9.99% (T), 1.88% (CORT), 8.87% (LH), and 10.4% (PRL).

Statistical Analyses

The specific breeding status of most sampled birds was unknown and data were, therefore, organized by calendar date rather than by stages of reproduction. For this, data were sorted by capture date and then divided into groups such that each time point included at least eight SY and eight ASY males. For each parameter, age- and capture date-related differences were analyzed using two-way analysis of variance (ANOVA) with age and date as main factors. When appropriate, ANOVAs were followed with Student–Newman–Keuls multiple pairwise comparison tests.

Data sets that did not comply with normality or equal variance criteria for ANOVA were either ranked (plasma T and CORT, testis masses, CP widths, and body masses) or transformed into square root (PRL) before analysis. Data that were ranked before ANOVA are presented as medians $\pm \frac{1}{2}$ interquartile intervals (Nicholson *et al.*, 1997). Other data are presented as means \pm standard deviations. We analyzed correlations between plasma T and CORT concentrations using Pearson product moment correlation coefficients. We compared the percentages of SY and ASY males undergoing replacement of primary flight feathers between July 1 and 10 and between July 11 and 20 using Fisher exact probability tests. Statistical significance level was in all cases set at $\alpha = 0.05$.

RESULTS

Plasma Hormone Concentrations

The concentrations of the four plasma hormones under study underwent highly significant seasonal changes (see ANOVA results, Table 1). Plasma concentrations of T were elevated shortly after arrival and during territory establishment (end of April–early May) and had decreased significantly by mid-May (Fig. 1). By the end of June, they were very low and by July, levels of this hormone were undetectable in most individuals. ASY males had seasonally higher plasma T concentrations than SY males (Table 1). Multiple comparison tests revealed that the two year-class groups differed at the time of arrival on breeding grounds (end of April), but not later.

CORT levels were highest at the beginning of the breeding season (early May) and, as was the case for T, had decreased significantly by mid-May. A further statistically significant decrease occurred between mid-June and July (Fig. 1). Levels did not differ between SY and ASY juncos (Table 1) and there was no age \times date interaction. Concentrations of plasma CORT and T were positively correlated in SY, as well as in ASY males (Fig. 2).

Plasma LH was high and did not vary statistically between April and June, but was significantly lower in July than during the 2 previous months (Fig. 3). Circulating concentrations of this hormone did not differ between SY and ASY males (Table 1).

TABLE 1

Results of Two-Way Analyses of Variance (ANOVA) Indicating the Effects of Age and Time Plus the Interaction between These Factors on Circulating Concentrations of Four Hormones, Testis Masses, Cloacal Protuberance Widths, and Body Masses of Adult Male Dark-Eyed Juncos Sampled from a Free-Living Population in Interior Alaska during Their Reproductive Period

Parameter	ANOVA		
	Age	Date	Interaction
Circulating hormone concentrations			
Testosterone	$F_{1,231} = 4.99$ $P = 0.027$	$F_{10,231} = 61.41$ $P < 0.001$	$F_{10,231} = 0.94$ n.s.
Corticosterone	$F_{1,185} = 1.78$ n.s.	$F_{7,185} = 17.97$ $P < 0.001$	$F_{7,185} = 0.93$ n.s.
Luteinizing hormone	$F_{1,197} = 0.19$ n.s.	$F_{8,197} = 22.18$ $P < 0.001$	$F_{8,197} = 1.37$ n.s.
Prolactin	$F_{1,216} = 2.89$ n.s.	$F_{7,216} = 93.70$ $P < 0.001$	$F_{7,216} = 2.22$ $P = 0.03$
Testis mass	$F_{1,181} = 10.86$ $P = 0.001$	$F_{8,181} = 24.47$ $P < 0.001$	$F_{8,181} = 1.44$ n.s.
Cloacal protuberance width			
	$F_{1,253} = 38.97$ $P < 0.001$	$F_{11,253} = 81.92$ $P < 0.001$	$F_{11,253} = 0.89$ n.s.
Body mass			
	$F_{1,252} = 1.04$ n.s.	$F_{11,252} = 4.82$ $P < 0.001$	$F_{11,252} = 0.79$ n.s.

Note. For each parameter, the calculated F values and corresponding degrees of freedom and probabilities are shown; n.s. (nonsignificant) indicates $P > 0.05$.

Circulating PRL concentrations were low shortly after arrival in early May, started to increase within 10 days (May 8 vs May 16: $P < 0.05$), were highest in June, and then started to decrease (Fig. 3). Seasonal changes in plasma PRL concentrations did not exhibit an overall year-class difference (age effect: $P = 0.09$), but there was a year class \times date interaction (Table 1). In July, but not earlier in the breeding season, ASY males had higher plasma PRL concentrations than SY males (Student–Newman–Keuls tests: $P < 0.05$).

Reproductive Morphology

Testis masses and CP sizes generally followed a similar seasonal time course of changes (Fig. 4). Birds had partially developed testes upon reaching their breeding area (average weight, April 25–May 3: 109 ± 47 mg ($n = 17$), compared to less than 5 mg in fully photoregressed males (Deviche *et al.*, 2000)). Testis masses more than tripled during the following 5 weeks and remained high until the end of June. At this

time, testes rapidly involuted, decreasing in mass from 309 ± 102 mg ($n = 21$) to 85 ± 110 mg ($n = 19$) between July 1 and 14. CPs were undeveloped (i.e., similar to those of nonphotostimulated males: Deviche *et al.*, 2000) on arrival and then rapidly increased in size for the next 6 weeks. They remained large until the end of June before rapidly involuting in July.

ASY juncos had seasonally heavier testes than SY males (Table 1). As shown by multiple comparison tests, the two groups of males differed at one time during gonadal recrudescence (mid-May) as well as immediately before regression (early July). ASY males also had larger CPs than SY males. CP sizes differed between the two year-classes during gonadal recrudescence (May) as well as at the end of the reproductive season (end of June to mid-July).

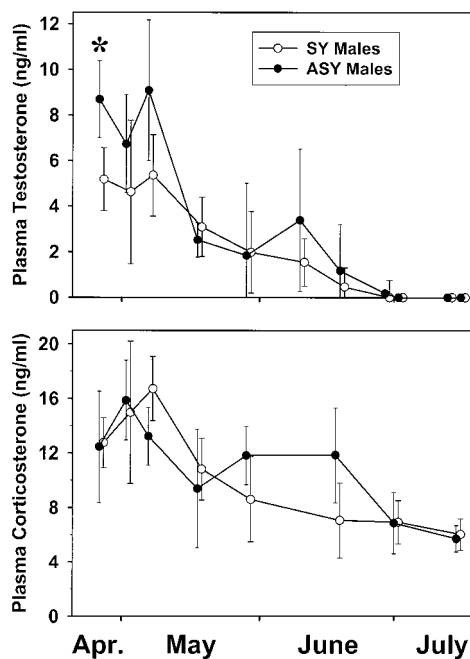


FIG. 1. Top: Seasonal changes in plasma testosterone concentrations (medians $\pm \frac{1}{2}$ interquartile intervals; $n \geq 8$ per point) in free-living interior Alaska (65°N ; 148°W) adult male Dark-eyed Juncos (*Junco hyemalis*) belonging to two age classes: second-year (SY; males hatched the previous year and entering their first breeding season) and after-second-year (ASY; males that are at least 2 years old). Bottom: Seasonal changes in baseline plasma corticosterone concentrations (medians $\pm \frac{1}{2}$ interquartile intervals; $n \geq 8$ per point) in two age classes of adult male Dark-eyed Juncos. *Denotes a significant difference between the two age groups ($P < 0.05$; Student–Newman–Keuls multiple comparison test).

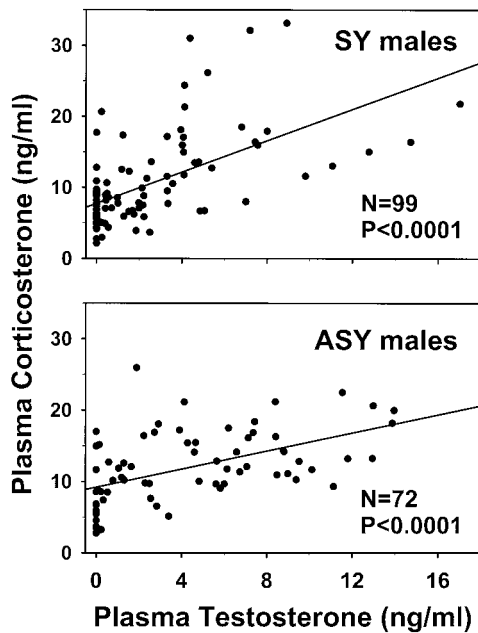


FIG. 2. Linear correlation between plasma testosterone and baseline corticosterone concentrations in two age classes of adult male Dark-eyed Juncos. Each point represents one individual. Probabilities (P s) were calculated from Pearson product moment correlation coefficients. See legend of Fig. 1 for additional information.

Molt

Prebasic molt started at the end of June, as indicated by the fact that 18 males (SY, $n = 10$; ASY, $n = 8$) caught between June 20 and 29 were not molting, but 3 of 8 males sampled on June 30 had lost at least one primary. Between July 1 and 10, 9 of 19 (47%) SY, but only 1 of 14 (7%) ASY males had initiated primary molt ($P = 0.02$). Similarly, more SY (19 of 19: 100%) than ASY (5 of 10: 50%) juncos had lost at least one primary between July 11 and 20 ($P = 0.002$).

Body Mass and Wing Chord

Body masses underwent highly significant seasonal changes (Table 1, Fig. 5). They were lowest during the weeks following arrival, then increased during the second half of May (May 23 vs May 13: $P < 0.05$), and remained relatively constant thereafter. SY and ASY males had similar masses throughout the study period. Thus, seasonal changes and age-related differences in reproductive tissues were not due to overall changes and differences in body mass.

DISCUSSION

The present investigation reports seasonal changes in plasma hormone concentrations and reproductive activity in free-living adult male juncos and demonstrates differences in some of these parameters, as well as in the timing of prebasic molt, in males experiencing their first cycle of gonadal development and regression and males that have undergone at least one such cycle. At the beginning of the breeding season, SY males had lower plasma T concentrations than ASY males. SY males also had seasonally smaller testes and CPs than ASY males. At the end of the breeding season, plasma PRL concentrations decreased earlier in SY males, and these males initiated prebasic molt earlier than ASY males. In contrast, seasonal changes in plasma LH and CORT concentrations and in body masses were year-class independent. What factors may be responsible for the observed hormonal and

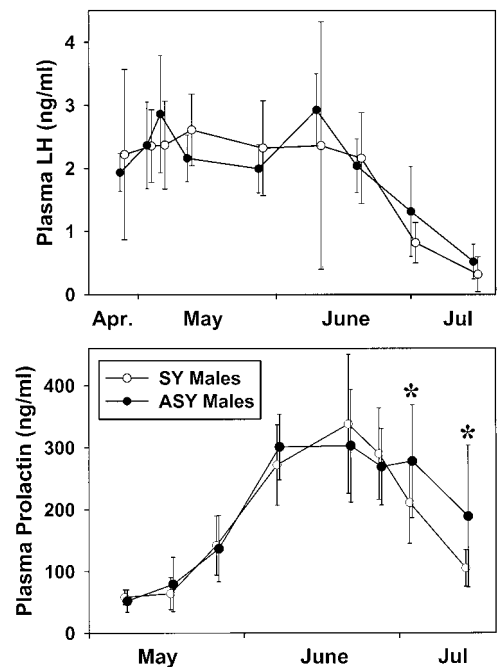


FIG. 3. Top: Seasonal changes in plasma concentrations (medians $\pm \frac{1}{2}$ interquartile intervals; $n \geq 8$ per point) of luteinizing hormone (LH) in two age classes of adult male Dark-eyed Juncos. Bottom: Seasonal changes in plasma prolactin concentrations (means \pm standard deviations; $n \geq 8$ per point, except for ASY males, June 11–20 ($n = 4$)) in two age classes of adult male Dark-eyed Juncos. See legend of Fig. 1 for additional information.

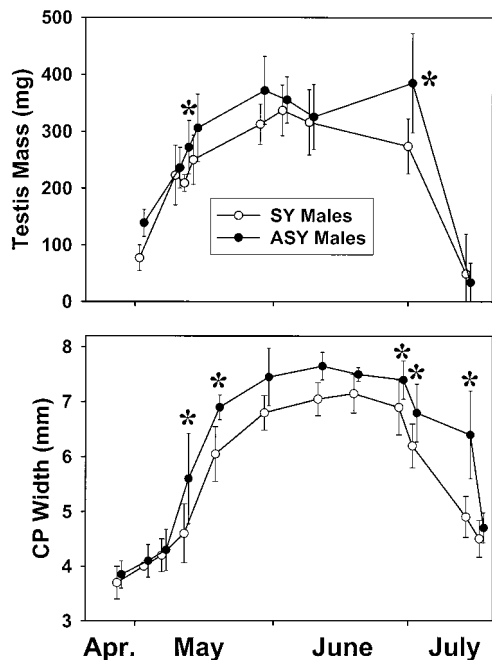


FIG. 4. Top: Seasonal changes in paired testis masses (medians \pm $\frac{1}{2}$ interquartile intervals; $n \geq 8$ per point) in two age classes of adult male Dark-eyed Juncos. Bottom: Seasonal changes in cloacal protuberance (CP) widths (medians \pm $\frac{1}{2}$ interquartile intervals; $n \geq 8$ per point) in two age classes of adult male Dark-eyed Juncos. See legend of Fig. 1 for additional information.

morphological differences between the two age classes?

Before addressing this issue, it should be noted that we do not know whether the observed differences between SY and ASY males resulted from an intrinsic effect of age on the reproductive system or from an influence of previous experience on the responsiveness of this system to environmental (e.g., territory size or quality) and/or social (e.g., mated vs not mated) factors. In a long-lived species (Common Tern, *Sterna hirundo*), plasma T gradually increases as a function of age in breeding, but relatively young males, whereas it does not exhibit marked age-related changes in older males (Nisbet *et al.*, 1999). In this species, therefore, the activity of the reproductive system may increase as a function of breeding experience until birds reach final maturation. At least some SY male juncos belonging to our study population hold territories and breed successfully (personal observations), but neither the specific breeding status of the sampled males (e.g., territorial vs "floaters") nor their

social history was known. It is, therefore, possible that SY and ASY males differed with respect to age as well as breeding experience. The present study does not separate these two variables.

The two age groups had similar circulating LH concentrations throughout the breeding cycle, as was reported for White-crowned Sparrows (Morton *et al.*, 1990). In addition, and as noticed in other species (Wingfield and Farner, 1978a,b; Schwabl *et al.*, 1980; Dawson, 1983; Wingfield, 1984; Hegner and Wingfield, 1986; Morton *et al.*, 1990), changes in plasma T and LH concentrations followed a different seasonal time course, with T levels decreasing in May, but LH levels remaining high until the end of June. We speculate that plasma T levels decreased in June despite continued elevated plasma LH levels because the androgen was then confined to the testes and/or other tissues rather than released into the circulation, perhaps as a result of increased PRL levels (see below). This hypothesis is supported by research on the endocrine response of passerines to behavioral challenges such as simulated territorial intrusion. Plasma T concentrations in males can increase within minutes of this challenge (Wingfield, 1985b; Wingfield and Wada, 1989; Wingfield and Hahn, 1994; see review by Wingfield *et al.*, 1994). This increase is accompanied by elevated plasma LH concentrations and may play a role in final sperm maturation. However, in male Song Sparrows, *Melospiza melodia*, elevated LH levels resulting from an agonistic encounter do not precede, but follow, an initial increase in T levels (Wingfield and Wada, 1989). Thus, the initial increase in T levels in response to a behavioral challenge may result not from LH stimulation, but from the release of a pre-existing (possibly gonadal) pool of androgen. At the

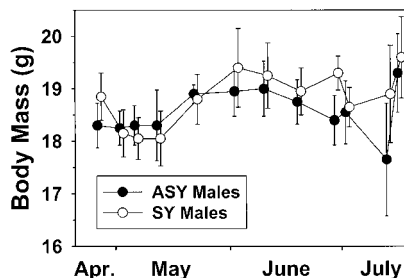


FIG. 5. Seasonal changes in body masses (medians \pm $\frac{1}{2}$ interquartile intervals; $n \geq 8$ per point) of two age classes of adult male Dark-eyed Juncos. See legend of Fig. 1 for additional information.

present time we do not know whether the androgen-secreting capacity of the testes differs between birds undergoing their first gonadal recrudescence and older birds, and the mechanism mediating a rapid, presumably LH-independent, activation of T secretion is not identified. Nevertheless, the year-class difference in plasma T concentrations that we observed was apparently not related to plasma LH levels.

Previous investigations demonstrated inhibitory effects of chronically elevated glucocorticoid levels on the avian reproductive system (Wilson and Follett, 1975; for review, see Deviche, 1983), but the present study provides no support for a role of adrenocortical function in the induction of differences in gonadal function in SY and ASY males. First, year-class differences in plasma T levels and testicular masses were not associated with differences in plasma CORT levels. Although baseline CORT levels were not year-class dependent, we cannot exclude the possibility of a year-class-related difference in stress response. In other species, this response varies as a function of physiological and ambient conditions (Schwabl *et al.*, 1991; Astheimer *et al.*, 1994; Wingfield *et al.*, 1994; Romero *et al.*, 1998a,b), but we do not know whether it is year-class dependent in adults. Second, plasma T and CORT concentrations decreased gradually during the breeding season and they were positively correlated with each other on an individual basis. This pattern resembles that described in other species (starling: Dawson and Howe, 1983; White-crowned Sparrow: Wingfield and Farner, 1978a,b; Snow Bunting, *Plectrophenax nivalis*: Romero and Wingfield, 1998c) and possibly resulted from androgen stimulation of glucocorticoid levels, as demonstrated previously in juncos (Klukowski *et al.*, 1997). Decreasing plasma CORT levels as the breeding season progresses may be of adaptive significance because administration of this hormone to breeding birds has detrimental effects on territorial and parental behavior (Pied Flycatcher, *Ficedula hypoleuca*: Silverin, 1986).

Prolactin treatment inhibits gonadal activity (Buntin and Tesch, 1985; Janik and Buntin, 1985; for review, see Sharp *et al.*, 1998). This hormone probably acts at multiple levels of the hypothalamo-pituitary-gonadal axis to promote the rapid involution of the reproductive system that takes place when birds become photorefractory (Dawson and Sharp, 1998; for reviews see Nicholls *et al.*, 1988 and Juss, 1993). Our results are

consistent with this conclusion, demonstrating that PRL levels were relatively low during gonadal recrudescence and highest in June, in advance of decreasing LH concentrations and gonadal involution. A contribution of circulating PRL to year-class differences in reproductive system activity is, however, unlikely because SY and ASY males differed with respect to their gonadal mass, plasma T concentrations, and CP sizes in May-June, but had different plasma PRL concentrations only in July. Furthermore, if the reproductive system activity of SY and ASY males differed as a result of plasma PRL-mediated inhibition, we would expect levels of this hormone to be higher in SY than in ASY males. This was not the case as plasma PRL was lower in SY than in ASY males in July. Thus, differences in reproductive function prior to gonadal regression were not related to PRL secretion.

There was a marked age difference in onset of prebasic molt, but the endocrine basis of this difference is currently unknown. It seems unlikely that this difference resulted from a year-class difference in the time elapsed after the previous molt because molt into the first adult plumage in hatching-year male juncos takes place at the same time (July to early September) as prebasic molt in adult males (Deviche, unpublished observations). Experimentally elevated T levels inhibit molt in juncos (Nolan *et al.*, 1992; Deviche, 1995) and other birds (Payne *et al.*, 1972). However, ASY males in the present study had higher plasma T levels than SY males only at the end of April, 2 months before molt started. This makes it unlikely that an age class difference in circulating concentrations of this steroid was the proximate factor responsible for the fact that SY males started molting earlier than ASY males. A recent investigation on starlings suggested that photoinduced PRL release is required for molt (Dawson and Sharp, 1998). In the present study, maximum plasma PRL concentrations were reached shortly before the beginning of molt, at which point they did not differ between SY and ASY juncos. In July, SY males had lower PRL levels, but started to molt earlier, than ASY males. Thus, there was no positive relationship between the onset of molt and the circulating PRL concentrations and our data do not support a role for PRL in the onset of prebasic molt in Dark-eyed Juncos.

The onset of prebasic molt is generally considered to indicate photorefractoriness (Nicholls *et al.*, 1988). Although CP sizes and testicular masses declined at the

same time in SY and ASY males, the molt data suggest the intriguing possibility that SY males became photorefractory earlier than ASY males despite the fact that all birds were exposed to identical environmental conditions of photoperiod and temperature while on the breeding grounds. During the breeding season, some male juncos apparently do not mate (Ketterson *et al.*, 1992) and, although at least some SY males nest successfully, more SY than ASY males may fail to obtain and hold a breeding territory. In other male songbirds, activity of the reproductive system is modulated by social influences, especially interactions with mates (Schwab and Lott, 1969; Moore, 1982; Dufty and Wingfield, 1986; Sorenson *et al.*, 1997; but see Silverin, 1991). Runfeldt and Wingfield (1985) reported that administration of estradiol to female Song Sparrows delays molt and maintains elevated circulating T levels for several weeks in their untreated mates. In Pied Flycatchers, males with an experimentally shortened brooding period had lower PRL concentrations than controls, although only females of this species normally brood (Silverin and Goldsmith, 1990). Thus, behavioral interactions (or a lack thereof) with females possibly contributed to mediating year-class differences in male juncos, especially at the end of the reproductive period. Consistent with this conclusion, captive SY and ASY male juncos that were not in contact with females showed largely similar physiological responses to artificial photostimulation (Crain *et al.*, in preparation) and photoinduced gonadal growth rates did not differ between captive SY and ASY White-crowned Sparrows (Lewis and Farner, 1973).

Chronically elevated T levels in males exert multiple behavioral and physiological effects, some of which apparently are beneficial (e.g., increased attractiveness to females, enhanced nest defense), while others are detrimental (compromised immune system, loss of fat reserves, inhibition of molt, decreased overwinter survival; Ketterson *et al.*, 1992, 1994; Casto *et al.*, 1999). At the present time, the long-term consequences, if any, of higher T levels only at the very beginning of the breeding period in ASY than in SY males are not known. Many male juncos are philopatric, returning to the same breeding territory from one year to the next (personal observations). Conceivably, familiarity with aspects of this territory results in ASY males having access to more and/or better food re-

sources and being able to allocate more energy to growth of reproductive tissues, including testes and secondary sexual characters such as cloacal protuberances, than SY males. Studies are fully warranted to evaluate this possibility and to determine whether differences in gonadal development between the two age classes result in age-specific effects on sperm production or fecundity.

ACKNOWLEDGMENTS

The authors are indebted to Cynthia Gullede, Cynthia Restrepo, Heidi Harbison, and Tanya Carlin for assistance in the field, to Renee Crain for comments on a draft of the manuscript, and to two anonymous reviewers for valuable suggestions for improvement of the original manuscript. We thank Lynn Eckermann, Simone Meddle, and Pete Wilson for expert assistance with corticosterone, luteinizing hormone, and prolactin assays, respectively. This work was partly supported by NIDCD Award No. KO1 DC00144 to P.D., NSF Award No. OPP-9530826 to J.C.W., and a BBSRC core strategic grant to P.J.S.

REFERENCES

- Astheimer, L. B., Buttemer, W. A., and Wingfield, J. C. (1994). Gender and seasonal differences in the adrenocortical response to ACTH challenge in an arctic passerine, *Zonotrichia leucophrys gambelii*. *Gen. Comp. Endocrinol.* **94**, 33–43.
- Ball, G. F., and Wingfield, J. C. (1987). Changes in plasma levels of luteinizing hormone and sex steroid hormones in relation to multiple-broodedness and nest-site density in male starlings. *Physiol. Zool.* **60**, 191–199.
- Bentley, G. E., Goldsmith, A. R., Dawson, A., Glennie, L. M., Talbot, R. T., and Sharp, P. J. (1997). Photorefractoriness in European Starlings (*Sturnus vulgaris*) is not dependent upon the long-day induced rise in plasma thyroxine. *Gen. Comp. Endocrinol.* **107**, 428–438.
- Buntin, J. D., and Tesch, D. (1985). Effects of intracranial prolactin administration on maintenance of incubation readiness, ingestive behavior, and gonadal condition in Ring Doves. *Horm. Behav.* **19**, 188–203.
- Casto, J. M., Ketterson, E. D., and Nolan, V., Jr. (1999). Elevated testosterone suppresses humoral and cell-mediated immunity in male dark-eyed juncos. [Abstract, 3rd Annual Meeting of the Society for Behavioral Neuroendocrinology, Charlottesville, VA]
- Dawson, A. (1983). Plasma gonadal steroid levels in wild starlings (*Sturnus vulgaris*) during the annual cycle and in relation to the stages of breeding. *Gen. Comp. Endocrinol.* **49**, 286–294.

- Dawson, A. (1997). Plasma-luteinizing hormone and prolactin during circannual rhythms of gonadal maturation and molt in male and female European starlings. *J. Biol. Rhythms* **12**, 371–377.
- Dawson, A., and Goldsmith, A. R. (1983). Plasma prolactin and gonadotrophins during gonadal development and the onset of photorefractoriness in male and female starlings (*Sturnus vulgaris*) on artificial photoperiods. *J. Endocrinol.* **97**, 253–260.
- Dawson, A., and Howe, P. D. (1983). Plasma corticosterone in wild starlings (*Sturnus vulgaris*) immediately following capture and in relation to body weight during the annual cycle. *Gen. Comp. Endocrinol.* **51**, 303–308.
- Dawson, A., and Sharp, P. J. (1998). The role of prolactin in the development of reproductive photorefractoriness and postnuptial molt in the European starling (*Sturnus vulgaris*). *Endocrinology* **139**, 485–490.
- Delville, Y., Sulon, J., Hendrick, J.-C., and Balthazart, J. (1984). Effect of the presence of females on the pituitary–testicular activity in male Japanese Quail (*Coturnix coturnix japonica*). *Gen. Comp. Endocrinol.* **55**, 295–305.
- Deviche, P. (1983). Interactions between adrenal function and reproduction in birds. In “Avian Endocrinology: Environmental and Ecological Perspectives” (S. Mikami *et al.*, Eds.), pp. 243–254. Japan Sci. Soc. Press, Tokyo/Springer-Verlag, Berlin.
- Deviche, P. (1995). Androgen regulation of avian premigratory hyperphagia and fattening: From ecophysiology to neuroendocrinology. *Am. Zool.* **35**, 234–245.
- Deviche, P., and Gullledge, C. C. (1999). Vocal control region sizes of an adult female songbird change seasonally in the absence of detectable circulating testosterone concentrations. *J. Neurobiol.* **42**, 202–211.
- Deviche, P., Saldanha, C. J., and Silver, R. (2000). Changes in brain gonadotropin releasing hormone- and vasoactive intestinal polypeptide-like immunoreactivity accompanying re-establishment of photosensitivity in male dark-eyed juncos (*Junco hyemalis*). *Gen. Comp. Endocrinol.* **117**, 8–19.
- Dufty, A. M., Jr., and Wingfield, J. C. (1986). The influence of social cues on the reproductive endocrinology of male Brown-headed Cowbirds: Field and laboratory studies. *Horm. Behav.* **20**, 222–234.
- Engels, W. L., and Jenner, C. E. (1956). The effect of temperature on testicular recrudescence in juncos at different photoperiods. *Biol. Bull.* **110**, 129–137.
- Follett, B. K., Farner, D. S., and Mattocks, P. W., Jr. (1975). Luteinizing hormone in plasma of White-crowned Sparrows, *Zonotrichia leucophrys gambelii*, during artificial photostimulation. *Gen. Comp. Endocrinol.* **26**, 126–134.
- Follett, B. K., Scanes, C. G., and Cunningham, F. J. (1972). A radioimmunoassay for avian luteinizing hormone. *J. Endocrinol.* **52**, 359–378.
- Hegner, R. E., and Wingfield, J. C. (1986). Behavioral and endocrine correlates of multiple brooding in the semicolonial House Sparrow *Passer domesticus*. I. Males. *Horm. Behav.* **20**, 294–312.
- Janik, D. S., and Buntin, J. D. (1985). Behavioural and physiological effects of prolactin in incubating ring doves. *J. Endocrinol.* **105**, 201–209.
- Juss, T. S. (1993). Neuroendocrine and neural changes associated with the photoperiodic control of reproduction. In “Avian Endocrinology” (P. J. Sharp, Ed.), pp. 47–60. Soc. Endocrinol., Bristol, UK.
- Ketterson, E. D., and Nolan, V., Jr. (1992). Hormones and life histories: An integrative approach. *Am. Nat.* **140**, S33–S62.
- Ketterson, E. D., Nolan, V., Jr., Wolf, L., and Goldsmith, A. R. (1990). Effect of sex, stage of reproduction, season, and mate removal on prolactin in Dark-eyed Juncos. *Condor* **92**, 922–930.
- Ketterson, E. D., Nolan, V., Jr., Wolf, L., and Ziegenfus, C. (1992). Testosterone and life histories: Effects of experimentally elevated testosterone on behavior and correlates of fitness in the Dark-eyed Junco (*Junco hyemalis*). *Am. Nat.* **140**, 980–999.
- Ketterson, E. D., Nolan, V., Jr., Cawthorn, M. J., Parker, P. G., and Ziegenfus, C. (1994). Phenotypic engineering: Using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. *Ibis* **138**, 70–86.
- Klukowski, L. A., Cawthorn, J. M., Ketterson, E. D., and Nolan, V., Jr. (1997). Effects of experimentally elevated testosterone on plasma corticosterone and corticosteroid-binding globulin in dark-eyed juncos (*Junco hyemalis*). *Gen. Comp. Endocrinol.* **108**, 141–151.
- Lewis, R. A., and Farner, D. S. (1973). Temperature modulation of photoperiodically induced vernal phenomena in White-crowned Sparrows (*Zonotrichia leucophrys*). *Condor* **75**, 279–286.
- Ligon, J. D. (1974). Green cones of the piñon pine stimulate late summer breeding in the Piñon Jay. *Nature* **250**, 80–81.
- Moore, M. C. (1982). Hormonal response of free-living male White-crowned Sparrows to experimental manipulation of female sexual behavior. *Horm. Behav.* **16**, 323–329.
- Morton, M. L., King, J. R., and Farner, D. S. (1969). Postnuptial and postjuvenile molt in White-crowned Sparrows in central Alaska. *Condor* **71**, 376–385.
- Morton, M. L., Peterson, L. E., Burns, D. M., and Allan, N. (1990). Seasonal and age-related changes in plasma testosterone levels in mountain White-crowned Sparrows. *Condor* **92**, 166–173.
- Nicholls, T. J., Goldsmith, A. R., and Dawson, A. (1988). Photorefractoriness in birds and comparison with mammals. *Physiol. Rev.* **68**, 133–176.
- Nicholson, M. C., Bowyer, R. T., and Kie, J. G. (1997). Habitat selection and survival of Mule Deer: Tradeoffs associated with migration. *J. Mammol.* **78**, 483–504.
- Nisbet, I. C. T., Finch, C. E., Thompson, N., Russeck-Cohen, E., Proudman, J. A., and Ottinger, M. A. (1999). Endocrine patterns during aging in the Common Tern (*Sterna hirundo*). *Gen. Comp. Endocrinol.* **114**, 279–286.
- Nolan, V., Jr., Ketterson, E. D., Ziegenfus, C., Cullen, D. P., and Chandler, C. R. (1992). Testosterone and avian life histories: Effects of experimentally elevated testosterone on prebasic molt and survival in male Dark-eyed Juncos. *Condor* **94**, 364–370.
- Parry, D. M., Goldsmith, A. R., Millar, R. P., and Glennie, L. M. (1997). Immunocytochemical localization of GnRH precursor in the hypothalamus of European Starlings during sexual maturation and photorefractoriness. *J. Neuroendocrinol.* **9**, 235–243.
- Payne, R. B., Farner, D. S., and King, J. R. (1972). Mechanisms and control of molt. In “Avian Biology” (D. S. Farner and J. R. King, Eds.), pp. 104–155. Academic Press, New York.
- Pyle, P. (1997). “Identification Guide to North American Birds. Part I. Columbidae to Ploceidae.” Slate Creek Press, Bolinas, CA.
- Romero, L. M., Soma, K. K., and Wingfield, J. C. (1998a). Changes in pituitary and adrenal sensitivities allow the snow bunting (*Plec-*

- trophenax nivalis*), an Arctic-breeding song bird, to modulate corticosterone release seasonally. *J. Comp. Physiol. [B]* **168**, 353–358.
- Romero, L. M., Soma, K. K., and Wingfield, J. C. (1998b). The hypothalamus and adrenal regulate modulation of corticosterone release in redpolls (*Carduelis flammea*)—An arctic-breeding songbird. *Gen. Comp. Endocrinol.* **109**, 347–355.
- Romero, L. M., Soma, K. K., O'Reilly, K. M., Suydam, R., and Wingfield, J. C. (1998c). Hormones and territorial behavior during breeding in snow buntings (*Plectrophenax nivalis*): An arctic-breeding songbird. *Horm. Behav.* **33**, 40–47.
- Runfeldt, S., and Wingfield, J. C. (1985). Experimentally prolonged sexual activity in female sparrows delays termination of reproductive activity in their untreated mates. *Anim. Behav.* **33**, 403–410.
- Sæther, B.-E. (1990). Age-specific variation in the reproductive performance of birds. *Current Ornithol.* **7**, 251–283.
- Saldanha, C. J., Deviche, P. J., and Silver, R. (1994). Increased VIP and decreased GnRH expression in photorefractory Dark-eyed Juncos (*Junco hyemalis*). *Gen. Comp. Endocrinol.* **93**, 128–136.
- Schwab, R. G., and Lott, D. F. (1969). Testis growth and regression in starlings (*Sturnus vulgaris*) as a function of the presence of females. *J. Exp. Zool.* **171**, 39–42.
- Schwabl, H., Bairlein, F., and Gwinner, E. (1991). Basal and stress-induced corticosterone levels of Garden Warblers, *Sylvia borin*, during migration. *J. Comp. Physiol. B. Biochem. Syst. Environ. Physiol.* **161**, 576–580.
- Schwabl, H., and Farner, D. S. (1989). Endocrine and environmental control of vernal migration in male White-crowned Sparrows, *Zonotrichia leucophrys gambelii*. *Physiol. Zool.* **62**, 1–10.
- Schwabl, H., Wingfield, J. C., and Farner, D. S. (1980). Seasonal variation in plasma levels of luteinizing hormone and steroid hormones in the European Blackbird *Turdus merula*. *Vogelwarte* **30**, 283–294.
- Sharp, P. J., Dawson, A., and Lea, R. W. (1998). Control of luteinizing hormone and prolactin secretion in birds. *Comp. Biochem. Physiol. [C]* **119C**, 275–282.
- Silverin, B. (1986). Corticosterone-binding proteins and behavioral effects of high plasma levels of corticosterone during the breeding period in the Pied Flycatcher. *Gen. Comp. Endocrinol.* **64**, 67–74.
- Silverin, B. (1991). Behavioral, hormonal, and morphological responses of free-living male Pied Flycatchers to estradiol treatment of their mates. *Horm. Behav.* **25**, 38–56.
- Silverin, B., and Goldsmith, A. R. (1990). Plasma prolactin concentrations in breeding Pied Flycatchers (*Ficedula hypoleuca*) with an experimentally prolonged brooding period. *Horm. Behav.* **24**, 104–113.
- Silverin, B., and Westin, J. (1995). Influence of the opposite sex on photoperiodically induced LH and gonadal cycles in the Willow Tit (*Parus montanus*). *Horm. Behav.* **29**, 207–215.
- Silverin, B., Kikuchi, M., and Ishii, S. (1997). Seasonal changes in follicle-stimulating hormone in free-living Great Tits. *Gen. Comp. Endocrinol.* **108**, 366–373.
- Sorenson, L. G., Nolan, P. M., Brown, A. M., Derrickson, A. M., and Monfort, S. L. (1997). Hormonal dynamics during mate choice in the northern pintail: A test of the challenge hypothesis. *Anim. Behav.* **54**, 1117–1133.
- Stokkan, K.-A., Sharp, P. J., and Unander, S. (1986). The annual breeding cycle of the high-arctic Svalbard Ptarmigan (*Lagopus mutus hyperboreus*). *Gen. Comp. Endocrinol.* **61**, 446–451.
- Wilson, F. E., and Follett, B. K. (1975). Corticosterone induced gonadosuppression in photostimulated Tree Sparrows. *Life Sci.* **17**, 1451–1456.
- Wingfield, J. C. (1984). Environmental and endocrine control of reproduction in the Song Sparrow, *Melospiza melodia*. I. Temporal organization of the breeding cycle. *Gen. Comp. Endocrinol.* **56**, 406–416.
- Wingfield, J. C. (1985a). Influences of weather on reproductive function in female Song Sparrows, *Melospiza melodia*. *J. Zool., Lond.* **205**, 545–558.
- Wingfield, J. C. (1985b). Short-term changes in plasma levels of hormones during establishment and defense of a breeding territory in male Song Sparrows, *Melospiza melodia*. *Horm. Behav.* **19**, 174–187.
- Wingfield, J. C., and Farner, D. S. (1978a). The annual cycle of plasma irLH and steroid hormones in feral populations of the White-crowned Sparrow, *Zonotrichia leucophrys gambelii*. *Biol. Reprod.* **19**, 1046–1056.
- Wingfield, J. C., and Farner, D. S. (1978b). The endocrinology of a natural breeding population of the White-crowned Sparrow (*Zonotrichia leucophrys pugetensis*). *Physiol. Zool.* **51**, 188–205.
- Wingfield, J. C., and Farner, D. S. (1979). Some endocrine correlates of renesting after loss of clutch or brood in the White-crowned Sparrow, *Zonotrichia leucophrys gambelii*. *Gen. Comp. Endocrinol.* **38**, 322–331.
- Wingfield, J. C., Follett, B. K., Matt, K. S., and Farner, D. S. (1980). Effect of day length on plasma FSH and LH in castrated and intact White-crowned Sparrows. *Gen. Comp. Endocrinol.* **42**, 464–470.
- Wingfield, J. C., and Goldsmith, A. R. (1990). Plasma levels of prolactin and gonadal steroids in relation to multiple-brooding and renesting in free-living populations of the Song Sparrow, *Melospiza melodia*. *Horm. Behav.* **24**, 89–103.
- Wingfield, J. C., and Hahn, T. P. (1994). Testosterone and territorial behaviour in sedentary and migratory sparrows. *Anim. Behav.* **47**, 77–89.
- Wingfield, J. C., and Wada, M. (1989). Changes in plasma levels of testosterone during male–male interactions in the Song Sparrow, *Melospiza melodia*: Time course and specificity of response. *J. Comp. Physiol. A. Sensory Neural Behav. Physiol.* **166**, 189–194.
- Wingfield, J. C., Smith, M. J., and Farner, D. S. (1982). Endocrine responses of White-crowned Sparrows to environmental stress. *Condor* **84**, 399–409.
- Wingfield, J. C., Moore, M. C., and Farner, D. S. (1983). Endocrine responses to inclement weather in naturally breeding populations of White-crowned Sparrows (*Zonotrichia leucophrys pugetensis*). *Auk* **100**, 56–62.
- Wingfield, J. C., Vleck, C. M., and Moore, M. C. (1992). Seasonal changes of the adrenocortical response to stress in birds of the Sonoran Desert. *J. Exp. Zool.* **264**, 419–428.
- Wingfield, J. C., Whaling, C. S., and Marler, P. (1994). Communication in vertebrate aggression and reproduction: The role of hormones. In "The Physiology of Reproduction" (E. Knobil and J. D. Neill, Eds.), 2nd ed., pp. 303–342. Raven Press, New York.