Seasonal and population variation in male testosterone levels in breeding orange-crowned warblers (*Vermivora celata*)

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ABSTRACT

Comparative hormone studies can reveal how physiology underlies life history variation. Here, we examined seasonal variation in plasma testosterone concentration between populations of male orange-crowned warblers (*Vermivora celata*) breeding in Fairbanks, Alaska (*V. c. celata*) and on Santa Catalina Island, California (*V. c. sordida*). These populations face different ecological constraints and exhibit different life histories. Alaska birds have a short breeding season, low annual adult survival, and high reproductive rates. In contrast, Catalina Island birds exhibit high adult survival and low reproductive rates despite having a long breeding season. We examined seasonal variation in male testosterone concentrations as a potential mechanism underlying differences in male reproductive strategies between populations. From 2006 to 2008, we sampled males during the pre-incubation, incubation, and nestling stages. Alaska males exhibited a seasonal testosterone pattern typical of northern passerines: testosterone levels were high during pre-incubation and declined during incubation to low levels during nestling provisioning. Testosterone concentrations in Catalina Island males, however, did not vary consistently with breeding stage, remained elevated throughout the breeding season, and were higher than in Alaska males during the nestling stage. We hypothesize that in Alaska, where short seasons and high adult mortality limit breeding opportunities, the seasonal testosterone pattern facilitates high mating effort prior to incubation, but high parental investment during the nestling stage. On Catalina Island, elevated testosterone levels may reflect the extended mating opportunities and high population density facing males in this population. Our results suggest that population variation in seasonal testosterone patterns in orange-crowned warblers may be a function of differences in life history strategy and the social environment.

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1. Introduction

Hormones are hypothesized to provide a mechanistic basis for life history variation in vertebrates (Sinervo and Svensson, 1998; Ricklefs and Wikelski, 2002; Ketterson and Nolan, 1999; Hau, 2007). In particular, the steroid hormone testosterone is regarded as a prime mediator of life history trade-offs between parental care and mating effort, or between reproduction and survival (Ketterson and Nolan, 1992; Wingfield et al., 2001; Hau, 2007). The behavioral and physiological effects of testosterone in male birds have been well studied, as have avian life histories. Thus, birds provide model systems for studying testosterone's role in mediating life history differences (Ricklefs and Wikelski, 2002). During the breeding season, high plasma testosterone concentrations facilitate mating effort in male birds by promoting singing (Silverin, 1980; Ketterson et al., 1992), courtship (Endstrom et al.,

1997; Fusani, 2008), territorial aggression (Beletsky et al., 1995; Wingfield et al., 2000), mate guarding (Moore, 1984; Saino and MØller, 1995), and pursuit of extra-pair matings (Ketterson et al., 1992; Chandler et al., 1994; Raouf et al., 1997). Correlative and experimental evidence also indicate that higher testosterone concentrations can lead to increased mating success (Moss et al., 1994; Alatalo et al., 1996; Raouf et al., 1997; Reed et al., 2006). However, males with high testosterone concentrations appear to invest in mating effort at the expense of parental care (e.g., McGl-othlin et al., 2007). Testosterone implants can reduce nestling provisioning (Silverin, 1980; Ketterson et al., 1992; Beletsky et al., 1995; Peters et al., 2002) and nest defense (Cawthorn et al., 1998) by males, often resulting in a reduction in the number or quality of offspring fledged (Silverin, 1980; Hegner and Wingfield, 1987; Beletsky et al., 1995; Reed et al., 2006). Thus, when mating opportunities overlap with offspring care, testosterone can mediate trade-offs between mating success and fledging success.

Although elevated testosterone concentrations have potential fitness benefits (e.g., increased annual reproductive success; Moss

et al., 1994; Reed et al., 2006), they can be costly in terms of reduced survival (Dufty, 1989; Nolan et al., 1992; Moss et al., 1994; Redpath et al., 2006; Reed et al., 2006). High testosterone concentrations can increase aggressive encounters that result in injury (Beletsky et al., 1995), or promote conspicuous behaviors that increase predation (Redpath et al., 2006). Elevated testosterone concentrations late in the breeding season can delay or prevent post-breeding molt (Runfeldt and Wingfield, 1985; Nolan et al., 1992; Dawson, 1994), thereby affecting survival in subsequent life history stages. High testosterone concentrations can also increase energy consumption (Wingfield, 1984; Ketterson et al., 1991; Buchanan et al., 2001; but, see Lynn et al., 2000; Buttemer et al., 2008), possibly limiting resources available for self-maintenance. Finally, testosterone may suppress immune function (Greives et al., 2006; Martin et al., 2008), either directly or indirectly by influencing the secretion of other hormones (Owen-Ashley et al., 2004; Poiani et al., 2000). This immunosuppressive effect, albeit not universal (Wingfield et al., 2001; Roberts et al., 2004), has led many to regard testosterone as a key mechanism underlying the trade-off between reproductive effort and survival (Folstad and Karter, 1992; Sheldon and Verhulst, 1996; Zera and Harshman, 2001; Hau, 2007).

Comparative field studies in a life history context can improve our understanding of how testosterone mediates male reproductive trade-offs (Hau, 2007). A particularly powerful approach is to control for phylogeny and compare seasonal variation in testosterone levels among populations of the same species that differ in life table variables such as annual survival and fecundity (Holberton and Dufty, 2005; Hau, 2007). Only a small number of studies, however, have examined population differences in seasonal testosterone patterns among conspecifics, or even congeners, that exhibit substantial life history variation (Wingfield et al., 1990; Moore et al., 2002; Van Roo et al., 2003; Goymann et al., 2006). Such efforts can elucidate the evolutionary linkage between testosterone and life history trade-offs (Hau, 2007).

Evidence is mounting that population differences in testosterone reflect life history strategy, but a number of extrinsic environmental factors may also explain population variation in testosterone levels. For example, male testosterone concentrations are often elevated in populations with higher breeding densities (Beletsky et al., 1990, 1992), possibly due to increased competition for limited resources such as food or mates (Wingfield et al., 1990; Parisot et al., 2005). Moreover, higher male testosterone concentrations are typical of mating systems with greater degrees of male–male aggression and lower levels of paternal care (reviewed in Wingfield et al., 1990). Thus, an understanding of how environmental, social, and behavioral factors correlate with testosterone concentrations can elucidate the extent to which population variation in hormone levels relates to life history differences.

We examined seasonal patterns of male testosterone concentrations in two populations of a passerine bird, the orange-crowned warbler (*Vermivora celata*), breeding near the species' northern range limit in central Alaska and near its southern range limit on Santa Catalina Island, off the coast of southern California. These populations face diverse selection pressures and exhibit substantial variation in life history traits. Warblers breeding on Catalina Island have relatively high annual adult survival, low annual fecundity, and a long breeding season, while Alaska birds have relatively low annual adult survival, high annual fecundity, and a short breeding season. This system therefore provides an opportunity for examining the relationship between testosterone and intraspecific variation in male life history strategies.

Life history theory predicts that populations with low adult survival should invest heavily in each reproductive attempt and relatively less in self-maintenance, whereas populations with high adult survival should invest relatively more in self-maintenance

and less in reproduction (Stearns, 1992; Ghalambor and Martin, 2001; Roff, 2002). Thus, if testosterone patterns reflect life history strategy, we expected that Alaska and Catalina Island males would exhibit different seasonal testosterone concentration profiles during breeding. Specifically, we predicted that testosterone profiles in Alaska males would reflect a high investment in both mating effort and parental care: high testosterone levels during the female fertile period and low levels during parental care. For Catalina Island males, we predicted that testosterone levels would remain low throughout the majority of the long breeding season to protect against testosterone-mediated costs to survival. Alternatively, seasonal testosterone patterns in one or both of these populations could be a function of factors other than life history strategy. Thus, in addition to life history traits, we consider how population differences in breeding density, mating opportunities, and behavior relate to seasonal testosterone patterns.

2. Methods

2.1. Study species

The orange-crowned warbler is an insectivorous wood warbler (family Parulidae) that breeds throughout western and northern North America. This open-cup nesting species is socially monogamous and exhibits biparental care (Sogge et al., 1994). We investigated two subspecies, *V. c. sordida* and *V. c. celata*, that breed at opposite ends of the species range. At the southern end is *V. c. sordida*, which is largely endemic to the California Channel Islands. Some *V. c. sordida* are year-round residents on the islands, but most spend the July–January non-breeding season along the coasts of southern California and northern Baja (Sogge et al., 1994). We studied *V. c. sordida* breeding in Bulrush Canyon on Santa Catalina Island (33°20′56″N; 118°26′59″W), hereafter referred to as the Catalina population. At the northern end is *V. c. celata*, which breeds in northern Canada and Alaska, and migrates thousands of kilometers to winter quarters principally along the U.S. Gulf Coast (Sogge et al., 1994). We studied breeding *V. c. celata* near Fairbanks, Alaska (64°47′41″N; 147°53′45″W), hereafter referred to as the Alaska population.

Our study of life history variation in this species is ongoing, but preliminary data show that the Catalina and Alaska populations face diverse selection pressures and exhibit substantial differences in life history traits (Table 1). Indeed, the life histories of these warblers vary along a gradient known as the slow–fast life history continuum (Ricklefs and Wikelski, 2002). Catalina birds have higher return rates, smaller clutch sizes, and higher nest predation rates than do Alaska birds. In most years, the relatively long Catalina breeding season allows for multiple renesting opportunities. Renesting is rare in Alaska birds due to a short breeding season.

Table 1

Life history data for orange-crowned warblers breeding on Santa Catalina Island, California (*V. c. sordida*) and in Fairbanks, Alaska (*V. c. celata*). Data were collected from 2003 to 2008 on Catalina Island, and from 2006 to 2008 in Alaska (Yoon et al., in prep; Sillett et al., in prep). Daily nest mortality probabilities were estimated with the maximum likelihood approach in Program MARK (White and Burnham, 1999; Alaska: $n = 57$, Catalina: $n = 326$; Sillett et al., in prep).

Population (subspecies)	Breeding season length	Annual male survival (proportion of males)	Clutch size	Breeding density	Daily nest mortality
Fairbanks, AK (*V. c. celata*)	50–60 days	0.40 ± 0.16	5–7 eggs	<1 pair/ha	0.2 ± 0.01
Catalina Island, CA (*V. c. sordida*)	100–120 days	0.65 ± 0.02	2–4 eggs	2–6 pairs/ha	0.4 ± 0.01

Catalina birds breed at densities that are much higher than those observed in Alaska (Table 1).

2.2. Sampling and hormone assays

We captured and sampled adult males during the 2006–2008 breeding seasons on Catalina (11 March–26 May) and in Alaska (17 May–5 July). Males were captured in mist nests either by luring them into nets with conspecific song recordings (playback) and a taxidermic mount, or by capturing them passively. Nets were observed from a distance so that capture time was known. Birds were captured between 06:30 and 14:00 on Catalina Island, and between 04:30 and 14:00 in Alaska. We recorded the amount of time that elapsed between the start of playback and capture ('playback time'; range: 2–37 min; mean ± SE: 8.5 min ± 0.76), and the time elapsed between capture and blood sampling ('bleed time'; range: 1–10 min; mean: 2.9 min ± 0.14). We acquired blood samples by puncturing the brachial vein with a 26-gauge hypodermic needle and collecting blood ($\sim$80 µl) into heparinized capillary tubes. After blood sampling, we recorded morphometrics and marked individuals with a U.S. Fish & Wildlife Service aluminum band and a unique combination of colored leg bands. Blood samples were kept on ice (<6 h) until centrifuged, after which plasma was removed and frozen until assayed.

Males were assigned to one of three breeding stages based on behavioral observations: pre-incubation, incubation, or nestling. The pre-incubation stage for a male began after territory establishment and lasted until his mate began incubating her first clutch. This period tended to be longer on Catalina (14–21 days) than in Alaska (10–14 days). The incubation and nestling stages are associated with first broods or early re-nests (Catalina only). Males were not sampled during second broods when this occurred in the Catalina population. Breeding stages were based on closely monitored nest chronology, and males were confirmed to be the social mate by repeated observations and/or nest video. Only males with known breeding stages were included in analyses.

We sampled a total of 123 known-stage males during the 2006–2008 breeding seasons (Alaska males: n = 73; Catalina male: n = 50). Breeding stage samples were collected for all years in Alaska, but only in 2006 and 2008 for Catalina (see Fig. 1 for sample sizes). A severe drought affected Catalina Island in the summer of 2007 and virtually no females nested. Only one female laid a two-egg clutch, but nestlings were abandoned before fledging (Langin et al., 2009). Thus, no Catalina males were sampled during incubation or nestling stages in 2007 and we did not include 2007 Catalina data in analyses.

Plasma was analyzed for testosterone concentration by direct radioimmunoassay following the procedures described in Wingfield et al. (1991) and Moore et al. (2000). Below, we refer to hormone levels as 'plasma testosterone concentrations' to be consistent with the bulk of the avian literature. However, we note that the testosterone antibody (WLI-T-3003S, Fitzgerald Industries, Concord, MA) used in this direct assay cross-reacts with dihydrotestosterone (DHT), and thus the assay measures total plasma androgens. Samples were run in two assays; intraassay variations were 9.1% and 11.5%, and interassay variation was 15.9%.

2.3. Statistics

We examined (1) variation in seasonal testosterone patterns between the two populations (population variation model), and (2) annual variation in seasonal testosterone patterns within each population (annual variation model). Separate analyses were necessary because we had seasonal hormone data for 3 years for Alaska, but only 2 years for Catalina. The population variation and annual variation models were similar in that plasma testoster-
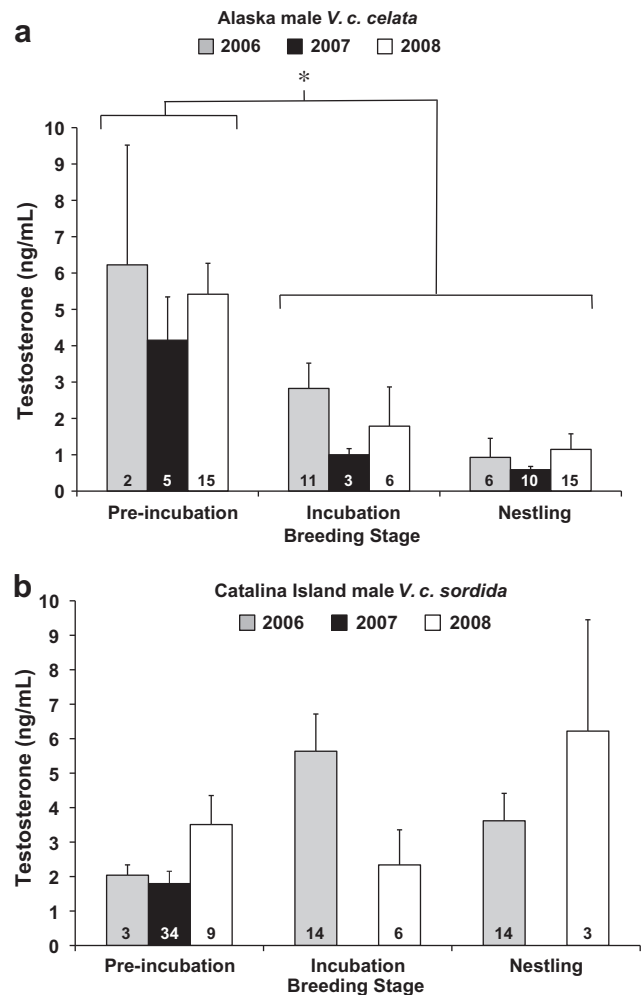

Fig. 1. Plasma testosterone concentrations (ng/mL) according to breeding stage in male orange-crowned warblers breeding a) in Fairbanks, Alaska (*V. c. celata*), and b) on Santa Catalina Island, California (*V. c. sordida*). Testosterone concentrations in Catalina males remained relatively high across breeding stages, while those of Alaska males declined significantly as breeding progressed. Note that extreme drought in 2007 caused reproductive failure on Catalina Island; thus, all male samples for that season on Catalina are 'pre-incubation.' Values are means ± 1 SE. Numbers in bars denote sample sizes.

one concentrations were analysed using a mixed-model ANCOVA with year and breeding stage as fixed main effects, and individual ID included as a random effect. The latter variable was incorporated in the model to account for the fact that some individuals were represented more than once in the data set (<20% of samples). In addition, both models included bleed time and body condition as covariates to account for these potential sources of variation in plasma testosterone concentrations. Body condition indices were calculated as the residuals of a mass versus tarsus length regression (R^2 = 0.14, P < 0.01). In addition to the above components, the population variation model included study site as a fixed main effect and a site*breeding stage interaction term, while the annual variation model included year*breeding stage and year*body condition interaction terms (Table 1). Annual variation analyses were conducted separately for each study site. Testosterone data were log transformed to meet model assumptions, but we used untransformed summary statistics in all figures. Random effects models were fit using the restricted maximum likelihood (REML) method. Post-hoc, pairwise comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test. All statistical analyses were performed using program JMP (SAS Institute 2009).

The models described above do not include playback time because not all birds were captured using target netting with song playback (i.e., some birds were captured by passive netting). Playback time had no significant effect on testosterone concentrations at either site (Alaska: $R^2 = 0.01$, $P = 0.49$; Catalina: $R^2 < 0.01$; $P = 0.88$), nor did we find an effect of capture method (target netting with song playback versus passive netting) on testosterone concentrations at either site (Alaska: $t_{1,70} = 1.35$, $P = 0.18$; Catalina: $t_{1,48} = 1.14$, $P = 0.26$).

3. Results

3.1. Population variation

Mean plasma testosterone concentrations and seasonal testosterone levels differed between sites (Table 2). Testosterone concentrations were higher overall in Catalina males (4.1 ± 0.48 ng/mL) than in Alaska males (2.6 ± 0.33 ng/mL), but did not differ significantly by year, bleed time, or body condition at either site (Table 2). Alaska and Catalina males exhibited different seasonal testosterone patterns (Table 2). In Alaska, male testosterone concentrations declined across breeding stages (Fig. 1a), such that testosterone concentrations were higher during pre-incubation than during the incubation (HSD, $P < 0.01$) and nestling stages (HSD, $P < 0.01$); concentrations in the incubation and nestling stages were not statistically different (HSD, $P = 0.27$). Plasma testosterone concentrations in Catalina males did not significantly differ between breeding stages (Fig. 1b). Average testosterone concentrations were statistically similar between populations during the pre-incubation and incubation stages (HSD, both $P > 0.10$), but higher in Catalina males than in Alaska males during the nestling stage (HSD, $P < 0.01$).

3.2. Annual variation

Seasonal testosterone patterns in both Alaska and Catalina males were consistent across years (Fig. 1, Table 2). Although incubation- and nestling-stage testosterone concentrations in Catalina

Table 2
Results from mixed-model ANCOVAs used to examine variation in plasma testosterone concentrations between (population variation model) and within (annual variation models) orange-crowned warbler populations breeding on Santa Catalina Island, California and in Fairbanks, Alaska. Each model included individual ID as a random effect and was fit using the restricted maximum likelihood (REML) method.

Source	df	F	P
Population variation model			
Site	1,80.31	6.52	**0.01**
Breeding stage	2,99.53	8.77	**<0.01**
Site∗breeding stage	2,100.4	10.53	**<0.01**
Year	2,98.54	2.39	0.10
Bleed time	1,105.5	2.28	0.13
Body condition ($n = 116$)	1,83.23	0.80	0.37
Annual variation model – Alaska			
Breeding stage	2,43.5	20.26	**<0.01**
Year	2,54.5	1.74	0.18
Breeding stage∗year	4,46.24	0.52	0.72
Body condition	1,47.79	0.44	0.51
Body condition∗year	2,55.03	0.80	0.45
Bleed time ($n = 70$)	1,54.87	2.32	0.13
Annual variation model – Catalina			
Breeding stage	2,23.72	0.17	0.84
Year	1,30.75	1.09	0.30
Breeding stage∗year	2,27.02	0.99	0.38
Body condition	1,7.387	1.72	0.23
Body condition∗year	1,28.78	4.00	0.06
Bleed time ($n = 46$)	1,23.26	1.13	0.30

Bold type highlights significant model effects ($P < 0.05$).

males appear different between the 2006 and 2008 breeding seasons (Fig. 1b), our data did not indicate a significant year effect or year∗breeding stage interaction after accounting for the variation in testosterone explained by the body condition∗year interaction (Table 2). We did not find a main effect of bleed time or body condition on male testosterone concentrations in either population using the annual variation model (Table 2).

4. Discussion

Male testosterone concentrations differed between two orange-crowned warbler subspecies breeding in Fairbanks, Alaska (*V. c. celata*) and on Catalina Island, California (*V. c. sordida*). Specifically, Alaska and Catalina males exhibited different seasonal testosterone profiles and different average testosterone concentrations during the nestling stage. This study adds to the small but growing body of work (Wingfield et al., 1990; Moore et al., 2002; Goymann et al., 2006) that addresses variation in seasonal testosterone patterns among conspecific populations with different life history strategies.

4.1. Testosterone in Alaska males

A short breeding season and low annual adult survival limit opportunities for Alaska males to achieve reproductive success. Thus, we predicted that Alaska males should invest heavily in both mating effort and parental care, and that testosterone concentrations should reflect these investments. Our results support this prediction: testosterone concentrations in Alaska males were high during pre-incubation and declined during incubation to low concentrations during nestling provisioning. This pattern is typical of biparental, socially monogamous passerines breeding at high latitudes (Wingfield et al., 1990; Hunt et al., 1995; Romero et al., 1998; Meddle et al., 2002).

High pre-incubation testosterone concentrations in Alaska males likely support an intense mating effort during the brief and highly synchronous female fertile period. Testosterone mediates behaviors that constitute mating effort, including singing, aggression, courtship, mate guarding, and pursuit of extra-pair matings. By facilitating mating effort, elevated testosterone concentrations can increase both within-pair and extra-pair mating success in male birds (Raouf et al., 1997; Reed et al., 2006). However, high testosterone concentrations can also reduce survival (Moss et al., 1994; Redpath et al., 2006; Reed et al., 2006). Further study is needed to determine if the testosterone pattern observed in Alaska males contributes to the lower annual survival exhibited by this population.

Alaska males down-regulated testosterone concentrations over the incubation period and maintained low concentrations during nestling provisioning (Fig. 1a). This change in testosterone concentrations should facilitate male parental investment and reduce testosterone-mediated fitness costs (Wingfield et al., 1990, 2001). Extra-pair mating opportunities rarely overlap with male parental care in this population due to the short breeding season, and the need for resource defense is probably reduced because food and nest sites are abundant for ground nesting passerines breeding at this latitude (Levy and Stiles, 1992). Thus, the benefits of high testosterone concentrations to aggression and mating effort during the nestling period are likely negligible. Elevated testosterone concentrations can, however, diminish male parental care and lead to lower fledging success (Silverin, 1980; Beletsky et al., 1995; Reed et al., 2006). Low testosterone concentrations during the nestling period may avert this potential cost, thereby enabling Alaska males to invest heavily in offspring care. Indeed, Alaska males exhibit

high parental effort and even provision their offspring at higher rates than do their mates (J. Yoon et al., in prep.).

4.2. Testosterone in Catalina males

A long breeding season and high annual adult survival provide Catalina males with multiple opportunities to gain reproductive success. According to theory, they should invest relatively less in each reproductive effort and more so in self-maintenance and survival (Stearns, 1992; Ghalambor and Martin, 2001; Roff, 2002). We predicted that Catalina males should maintain relatively low testosterone concentrations to protect against testosterone-mediated survival costs. Our results did not support this prediction because Catalina males had elevated testosterone concentrations during all breeding stages (Fig. 1b). This seasonal pattern is unusual for biparental, socially monogamous birds (see Wingfield et al., 1990), and may represent an adaptation to local conditions because Alaska males exhibited a typical testosterone profile.

The seasonal testosterone pattern (i.e., a prolonged elevation in testosterone) in Catalina males is probably due in part to the high breeding density of this population (Ball and Wingfield, 1987; Beletsky et al., 1990, 1992; Wingfield and Hahn, 1994). High densities can lead to a greater degree of male-male interactions and acute increases in testosterone concentrations; high testosterone concentrations in turn may facilitate competitive ability by increasing aggression (reviewed in Wingfield et al., 1990). This hypothesis is supported by the finding that, during incubation, Catalina males respond more aggressively to simulated territorial intrusion than do Alaska males (J. Yoon et al., in prep.).

Elevated testosterone concentrations in Catalina males during the incubation and nestling stages may also reflect a prolonged investment in mating effort. Frequent nest predation on Catalina (Peluc et al., 2008) leads to renesting from March–May or June in most years. This extended access to fertile females can prolong testosterone release in males (Moore, 1982, 1983). A prolonged elevation in testosterone can, in turn, sustain male breeding physiology and behavior (Silverin, 1980; Runfeldt and Wingfield, 1985; Nolan et al., 1992) and may enhance mate guarding or the ability to compete for extra-pair matings (e.g., Raouf et al., 1997; Reed et al., 2006). During the pre-incubation stage, elevated testosterone concentrations and a high mating effort may be expected, but high testosterone concentrations during incubation and nestling provisioning suggest that Catalina males may continue to invest substantially in extra-pair mating. We are currently measuring paternity rates in this population to test this hypothesis.

Prolonged elevations in testosterone concentrations, such as those exhibited by Catalina males through the nestling period, are expected to inhibit male parental care (e.g., Silverin, 1980; Ketterson et al., 1992; Beletsky et al., 1995; Peters et al., 2002) and reduce survival (Moss et al., 1994; Redpath et al., 2006; Reed et al., 2006). Yet, Catalina males provide substantial offspring care in the form of high nestling provisioning rates (J. Yoon et al., in prep), and they exhibit relatively high annual return rates (Table 1). This unexpected, and perhaps unique, relationship between testosterone and parental care, and between testosterone and survival, in Catalina males may provide valuable insight into the evolutionary linkage between testosterone and male reproductive trade-offs.

4.3. Testosterone and male reproductive trade-offs

Apparent conservation in the regulation of male traits by testosterone has prompted the 'evolutionary constraint hypothesis' (Ketterson and Nolan, 1999; Hau, 2007), which posits that testosterone and certain male traits are inseparably linked, such that the system (i.e., the hormone-signaling processes, target tissue responses, and trait expression) evolves as a unit. This hypothesis predicts that the behavioral effects of testosterone are consistent across species and populations (Ketterson and Nolan, 1999; Hau, 2007). The remarkably consistent effects of testosterone implants on male traits in myriad species support this prediction.

Recent evidence, however, indicates that male traits can be dissociated from control by testosterone in some populations (Van Duyse et al., 2000, 2002; Lynn et al., 2005; Lynn, 2008; Moore et al., 2004). For example, the extent to which testosterone controls aggression varies latitudinally in *Zonotrichia* sparrows (Moore, 1984; Wingfield and Hahn, 1994; Meddle et al., 2002; Moore et al., 2002, 2004). The degree to which testosterone inhibits paternal care also varies among members of the *Calcarius /Plectrophenax* clade (Hunt et al., 1999; Lynn et al., 2002, 2005). This variation in the control of male traits by testosterone supports an alternate explanation, the 'evolutionary potential hypothesis,' which states that selection shapes the specific linkages between testosterone and male traits, and that components of the system evolve independently (Finch and Rose, 1995; Sinervo and Svensson, 1998; Hau, 2007). This hypothesis predicts that the degree to which testosterone controls certain male traits can vary between populations with different life histories (Hau, 2007).

The population differences in seasonal testosterone concentration patterns observed in our comparative study of *V. celata* support the evolutionary potential hypothesis. Alaska males exhibited a seasonal testosterone concentration pattern consistent with predictions based on the expected (i.e., typical) costs and benefits of elevated testosterone levels. Catalina males, in contrast, had relatively high survival and substantial male parental care despite prolonged elevations in testosterone concentrations, which suggests that these traits may be dissociated from control by testosterone in this population. Further experimentation is needed to test this hypothesis, including manipulations of the seasonal testosterone pattern via hormone implants.

5. Conclusions

Male orange-crowned warblers from populations at opposite ends of the life history continuum had different patterns of testosterone during breeding. Testosterone concentrations in fast-end Alaska males peaked during the pre-incubation stage but declined to low levels during the nestling stage, a pattern typical of biparental, socially monogamous passerines. Slow-end Catalina males maintained relatively high testosterone concentrations across all breeding stages, a pattern that is apparently rare among small, temperate passerines. Seasonal testosterone patterns in Alaska fit predictions based on life history strategy whereas patterns on Catalina did not. Our results suggest that population variation in seasonal testosterone patterns may be a function of different social environments. The testosterone concentration patterns described herein provide an excellent basis for future experimentation. In particular, this system provides a promising opportunity for future studies aimed at testing competing hypotheses for the evolutionary linkage between testosterone and male reproductive trade-offs.

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References

Alatalo, R.V., Hoglund, J., Lundberg, A., Rintamaki, P.T., Silverin, B., 1996. Proceedings of the Royal Society B: Biological Sciences 263, 1697–1702.

Ball, G.F., Wingfield, J.C., 1987. Changes in plasma levels of sex steroids in relation to multiple broodedness and nest site density in male starlings. Physiological Zoology 60, 191–199.

Beletsky, L.D., Orians, G.H., Wingfield, J.C., 1990. Steroid hormones in relation to territoriality, breeding density, and parental behavior in male yellow-headed blackbirds. Auk 107, 60–68.

Beletsky, L.D., Orians, G.H., Wingfield, J.C., 1992. Year-to-year patterns of circulating levels of testosterone and corticosterone in relation to breeding density, experience, and reproductive success of the polygynous red-winged blackbird. Hormones and Behavior 26, 420–432.

Beletsky, L.D., Gori, D.F., Freeman, S., Wingfield, J.C., 1995. Testosterone and polygyny in birds. Current Ornithology 12, 654–662.

Buchanan, K.L., Evans, M.R., Goldsmith, A.R., Bryant, D.M., Rowe, L.V., 2001. Testosterone influences basal metabolic rate in male house sparrows: a new cost of dominance signaling? Proceedings of the Royal Society B: Biological Sciences 268, 1337–1344.

Buttemer, W.A., Warne, S., Bech, C., Astheimer, L.B., 2008. Testosterone effects on avian basal metabolic rate and aerobic performance. Facts and artifacts. Comparative Biochemistry and Physiology A 150, 204–210.

Cawthorn, J.M., Morris, D.L., Ketterson, E.D., Nolan Jr., V., 1998. Influence of experimentally elevated testosterone on nest defense in dark-eyed juncos. Animal Behaviour 56, 617–621.

Chandler, C.R., Ketterson, E.D., Nolan Jr., V., Ziegenfus, C., 1994. Effects of testosterone on spatial activity in free-ranging male dark-eyed juncos, Junco hyemalis. Animal Behaviour 47, 1445–1455.

Dawson, A., 1994. The effects of daylength and testosterone on the initiation and progress of moult in starlings, Sturnus vulgaris. Ibis 136, 335–340.

Dufty, A.M., 1989. Testosterone and survival. Hormones and Behavior 23, 185–193.

Endstrom, D.A., Ketterson, E.D., Nolan Jr., V., 1997. Testosterone and mate choice in the dark-eyed junco. Animal Behaviour 54, 1135–1146.

Finch, C.E., Rose, M.R., 1995. Hormones and the physiological architecture of testosterone's effects on fitness. American Naturalist 167, 667–683.

Folstad, I., Karter, A.J., 1992. Parasites, bright males, and the immunocompetence handicap. American Naturalist 139, 603–622.

Fusani, L., 2008. Testosterone control of male courtship in birds. Hormones and Behavior 54, 227–233.

Ghalambor, C.K., Martin, T.E., 2001. Fecundity-survival trade-offs and parental risk-taking in birds. Science 292, 494–497.

Goymann, W., Geue, D., Schwabl, I., Links, H., Schmidl, D., Schwabl, H., Gwinner, E., 2006. Testosterone and corticosterone during the breeding cycle of equatorial and European stonechats (Saxicola torquata axillaris and S. t. rubicola). Hormones and Behavior 50, 779–785.

Greives, T.J., McGlothlin, J.W., Jawor, J.M., Demas, G.E., Ketterson, E.D., 2006. Testosterone and innate immune function inversely covary in a wild population of breeding dark-eyed juncos (Junco hyemalis). Functional Ecology 20, 812–818.

Hau, M., 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. BioEssays 29, 133–144.

Hegner, R.E., Wingfield, J.C., 1987. Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. Auk 104, 462–469.

Holberton, R.L., Dufty Jr., A.M., 2005. Hormones and variation in life history strategies of migratory and non-migratory birds. In: Marra, P., Goldberg, R. (Eds.), Birds of Two Worlds: The Ecology and Evolution of Migratory Birds. Johns Hopkins Press, pp. 290–302.

Hunt, K., Wingfield, J.C., Astheimer, L.B., Buttemer, W.A., 1995. Temporal patterns of territorial behavior and circulating testosterone in the Lapland longspur and other arctic passerines. American Zoologist 35, 274–284.

Hunt, K.E., Hahn, T.P., Wingfield, J.C., 1999. Endocrine influences on parental care during a short breeding season: testosterone and male parental care in Lapland longspurs (Calcarius lapponicus). Behavioral Ecology and Sociobiology 45, 360–369.

Ketterson, E.D., Nolan Jr., V., Wolf, L., Ziegenfus, C., Dufty, A.M., Ball, G.F., Johnson, T.S., 1991. Testosterone and avian life histories: the effect of experimentally elevated testosterone on corticosterone and body mass in male Dark-eyed Juncos (Junco hyemalis). Hormones and Behavior 25, 489–503.

Ketterson, E.D., Nolan Jr., V., 1992. Hormones and avian life histories: an integrative approach. American Naturalist 140, S33–S62.

Ketterson, E.D., Nolan Jr., V., Wolf, L., Ziegenfus, C., 1992. Testosterone and avian life histories: effects of experimentally elevated testosterone on behavior and correlates of fitness in the dark-eyed junco (Junco hyemalis). American Naturalist 140, 980–999.

Ketterson, E.D., Nolan Jr., V., 1999. Adaptation, exaptation, and constraint: a hormonal perspective. American Naturalist 154, S4–S25.

Langin, K.M., Sillett, T.S., Yoon, J., Sofaer, H.R., Morrison, S.A., Ghalambor, C.K., 2009. Reproductive consequences of an extreme drought for orange-crowned warblers on Santa Catalina and Santa Cruz Islands. Proceedings of the 7th California Islands Symposium.

Levy, D.J., Stiles, F.G., 1992. Evolutionary precursors of long-distance migration: Resource availability and movement patterns in neotropical landbirds. American Naturalist 140, 447–476.

Lynn, A.M., Houtman, W.W., Weathers, E.D., Ketterson, V., Nolan, Jr., 2000. Testosterone increases activity but not daily energy expenditure in captive male dark-eyed juncos. Animal Behaviour 60, 581–587.

Lynn, S.E., Hayward, L.S., Benowitz-Fredericks, Z.M., Wingfield, J.C., 2002. Behavioural insensitivity to supplementary testosterone during the parental phase in the chestnut-collared longspur, Calcarius ornatus. Animal Behaviour 63, 795–803.

Lynn, S.E., Walker, B.G., Wingfield, J.C., 2005. A phylogenetically controlled test for hypotheses for behavioral insensitivity to testosterone in birds. Hormones and Behavior 47, 170–177.

Lynn, S.E., 2008. Behavioral insensitivity to testosterone: why and how does testosterone alter paternal behavior and aggressive behavior in some avian species but not others? General and Comparative Endocrinology 157, 233–240.

Martin, L.B., Weil, Z.M., Nelson, R.J., 2008. Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. Philosophical Tractions of the Royal Society B 363, 321–339.

Meddle, S.L., Romero, L.M., Astheimer, L.B., Buttemer, W.A., Moore, I.T., Wingfield, J.C., 2002. Steroid hormone interrelationships with territorial aggression in an arctic-breeding songbird, Gambel's white-crowned sparrow, Zonotrichia leucophrys gambelii. Hormones and Behavior 42, 212–221.

McGlothlin, J.W., Jawor, J.M., Ketterson, E.D., 2007. Natural variation in a testosterone-mediated trade-off between mating effort and parental effort. American Naturalist 170, 864–875.

Moore, M.C., 1982. Hormonal responses of free-living male white-crowned sparrows to experimental manipulation of female sexual behavior. Hormones and Behavior 16, 323–329.

Moore, M.C., 1983. Effect of female sexual displays on the endocrine physiology and behavior of male white crowned sparrows, Zonotrichia leucophrys gambelii. Journal of Zoology, London 199, 137–148.

Moore, M.C., 1984. Changes in territorial defense produced by changes in circulation levels of testosterone: a possible hormonal basis for mate-guarding behavior in white-crowned sparrows. Behaviour 88, 215–226.

Moore, I.T., Lerner, J.P., Lerner, D.T., Moore, R.T., 2000. Relationships between annual cycles of testosterone, corticosterone, and body condition in male red-spotted garter snakes, Thamnophis sirtalis concinnus. Physiological and Biochemical Zoology 73, 307–312.

Moore, I.T., Perfito, N., Wada, H., Sperry, T.S., Wingfield, J.C., 2002. Latitudinal variation in plasma testosterone levels in birds of the genus Zonotrichia. General and Comparative Endocrinology 129, 13–19.

Moore, I.T., Wada, H., Perfito, N., Busch, D.S., Hahn, T.P., Wingfield, J.C., 2004. Territoriality and testosterone in an equatorial population of rufous-collared sparrow, Zonotrichia capensis. Animal Behaviour 67, 411–420.

Moss, R., Parr, R., Lambin, X., 1994. Effects of testosterone on breeding density, breeding success and survival of red grouse. Proceedings of the Royal Society B 258, 175–180.

Nolan Jr., V., Ketterson, E.D., Ziegenfus, C., Cullen, D.P., 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.

Owen-Ashley, N.T., Hasselquist, D., Wingfield, J.C., 2004. Androgens and the immunocompetence handicap hypothesis: unraveling direct and indirect pathways of immunosuppression in song sparrows. American Naturalist 164, 490–505.

Parisot, M., Tanvez, A., Lacroix, A., Vallet, E., Béguin, N., Leboucher, G., 2005. Social competition and plasma testosterone profile in domesticated canaries: an experimental test of the challenge hypothesis. Hormones and Behavior 48, 225–232.

Peluc, S.I., Sillett, T.S., Rotenberry, J.T., Ghalambor, C.K., 2008. Adaptive phenotypic plasticity in an island songbird exposed to a novel predation risk. Behavioral Ecology 19, 830–835.

Peters, A., Cockburn, A., Cunningham, R., 2002. Testosterone treatment suppresses paternal care in superb fairy-wrens, Malurus cyaneus, despite their concurrent investment in courtship. Behavioral Ecology and Sociobiology 51, 538–547.

Poiani, A., Goldsmith, A.R., Evans, M.R., 2000. Ectoparasites of house sparrows (Passer domesticus): an experimental test of the immunocompetance handicap hypothesis and a new model. Behavioral Ecology and Sociobiology 47, 230–242.

Raouf, S.A., Parker, P.G., Ketterson, E.D., Nolan Jr., V., Ziegenfus, C., 1997. Testosterone affects reproductive success by influencing extra-pair fertilizations in male dark-eyed juncos (Aves: Junco hyemalis). Proceedings of the Royal Society B 264, 1599–1603.

Redpath, S.M., Mougeot, F., Leckie, F.M., Evans, S.A., 2006. The effects of autumn testosterone on survival and productivity in red grouse, Lagopus lagopus scoticus. Animal Behaviour 71, 1297–1305.

Reed, W.L., Clark, M.E., Parker, P.G., Raouf, S.A., Arguedas, N., Monk, D.S., Snajdr, E., Nolan Jr., V., Ketterson, E.D., 2006. Physiological effects on demography: a long-term experimental study of testosterone's effects on fitness. American Naturalist 167, 667–683.

Ricklefs, R.E., Wikelski, M., 2002. The physiology/life-history nexus. Trends in Ecology and Evolution 17, 462–468.

Roberts, M.L., Buchanan, K.L., Evans, M.R., 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. Animal Behaviour 68, 227–239.

Roff, D.A., 2002. Life history evolution. Sinauer Associates, Sunderland, MA.

Romero, L.M., Soma, K.K., O'Reilly, K.M., Suydam, R., Wingfield, J.C., 1998. Hormones and territorial behavior during breeding in snow buntings *Plectrophenax nivalis*: an arctic-breeding songbird. Hormones and Behavior 33, 40–47.

Runfeldt, S., Wingfield, J.C., 1985. Experimentally prolonged sexual activity in female sparrows delays termination of reproductive activity in their untreated mates. Animal Behaviour 33, 403–410.

Saino, N., Møller, A.P., 1995. Testosterone-induced depression of male parental behavior in the barn swallow: female compensation and effects on seasonal fitness. Behavioral Ecology and Sociobiology 33, 151–157.

Sheldon, B.C., Verhulst, S., 1996. Ecological immunity: costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology and Evolution. 11, 317–321.

Silverin, B., 1980. Effects of a long-acting testosterone treatment on free-living pied flycatchers, *Ficedula hypoleuca*. Animal Behaviour 28, 906–912.

Sinervo, B., Svensson, E., 1998. Mechanistic and selective causes of life history trade-offs and plasticity. Oikos 83, 432–442.

Sogge, M.K., Gilbert, W.M., Riper III, C.v., 1994. Orange-crowned Warbler (*Vermivora celata*). In: Poole, A., Gill, F. (Eds.), The Birds of North America, No. 101. Academy of Natural Sciences, Philadelphia, and American Ornithologists' Union, Washington, DC.

Stearns, S., 1992. The evolution of life histories. Oxford University Press, Oxford.

Van Duyse, E., Pinxten, R., Eens, M., 2000. Does testosterone affect the trade-off between investment in sexual/territorial behaviour and parental care in male great tits? Behaviour 137, 1503–1515.

Van Duyse, E., Pinxten, R., Eens, M., 2002. Effects of testosterone on song, aggression, and nestling feeding behavior in male great tits, *Parus major*. Hormones and Behavior 41, 178–186.

Van Roo, B.L., Ketterson, E.D., Sharp, P.J., 2003. Testosterone and prolactin in two songbirds that differ in paternal care: the blue-headed vireo and the red-eyed vireo. Hormones and Behavior 44, 435–441.

White, G.C., Burnham, K.P., 1999. Program MARK: survival estimation from populations of marked animals. Bird Study (Suppl. 46), 120–138.

Wingfield, J.C., 1984. Androgens and mating systems: testosterone-induced polygyny in normally monogamous birds. Auk 101, 665–671.

Wingfield, J.C., Hegner, R.E., Dufty Jr., A.M., Ball, G.E., 1990. The "challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. American Naturalist 136, 829–846.

Wingfield, J.C., Hegner, R.E., Lewis, D., 1991. Circulating levels of luteinizing hormone and steroid hormones in relation to social status in the cooperatively breeding white-browed sparrow weaver, *Plocepasser mahali*. Journal of Zoology, London 225, 43–48.

Wingfield, J.C., Hahn, T.P., 1994. Testosterone and territorial behaviour in sedentary and migratory sparrows. Animal Behaviour 47, 77–89.

Wingfield, J.C., Jacobs, J.D., Tramontin, A.D., Perfito, N., Meddle, S., Maney, D.L., Soma, K., 2000. Toward and ecological basis of hormone–behavior interactions in reproduction of birds. In: Wallen, K., Schneider, J. (Eds.), Reproduction in Context. MIT Press, Cambridge, pp. 85–128.

Wingfield, J.C., Lynn, S.E., Soma, K.K., 2001. Avoiding the 'costs' of testosterone: ecological bases of hormone–behavior interactions. Brain, Behavior and Evolution 57, 239–251.

Zera, A.J., Harshman, L.G., 2001. The physiology of life history trade-offs in animals. Annual Review of Ecology and Systematics 32, 95–126.