Physiological Effects on Demography: A Long-Term Experimental Study of Testosterone's Effects on Fitness

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Submitted April 27, 2005; Accepted November 22, 2005; Electronically published April 5, 2006

Online enhancement: appendix.

abstract: Understanding physiological and behavioral mechanisms underlying the diversity of observed life-history strategies is challenging because of difficulties in obtaining long-term measures of fitness and in relating fitness to these mechanisms. We evaluated effects of experimentally elevated testosterone on male fitness in a population of dark-eyed juncos studied over nine breeding seasons using a demographic modeling approach. Elevated levels of testosterone decreased survival rates but increased success of producing extra-pair offspring. Higher overall fitness for testosterone-treated males was unexpected and led us to consider indirect effects of testosterone on offspring and females. Nest success was similar for testosterone-treated and control males, but testosterone-treated males produced smaller offspring, and smaller offspring had lower postfledging survival. Older, more experienced females preferred to mate with older males and realized higher reproductive success when they did so. Treatment of young males increased their ability to attract older females yet resulted in poor reproductive performance. The higher fitness of testosterone-treated males in the absence of a comparable natural phenotype suggests that the natural phenotype may be constrained. If this phenotype were to arise, the negative social effects on offspring and mates suggest that these effects might prevent high-testosterone phenotypes from spreading in the population.

Keywords: dark-eyed junco, testosterone, fitness, extra-pair fertilization, age, growth rates.

Physiological mechanisms can orchestrate trade-offs among life-history characteristics (Stearns 1989; Ricklefs and Wikelski 2002; Zhao and Zera 2002), but understanding how physiology influences the evolution of life histories is challenging without accurate measures of both fitness and physiology under a realistic background of selection. Hormonal manipulations of individuals in free-living populations provide a powerful experimental tool for altering suites of physiological and behavioral characters in order to explore the consequences of these alterations under agents of natural selection present in the wild (Marler and Moore 1988; Ketterson et al. 1996; Zera and Harshman 2001; Ricklefs and Wikelski 2002). In birds, elevation of testosterone in free-living individuals results in changes in many traits correlated with fitness (reviewed in Ketterson and Nolan 1999). For example, elevated testosterone levels in male breeders are associated with increased song rates and attractiveness, as well as with higher levels of stress hormones and suppressed immune function (summarized in Ketterson and Nolan 1999; Casto et al. 2001). Collectively, such phenotypic consequences of elevated testosterone predict both positive and negative effects on various correlates of fitness, such as mating success

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Am. Nat. 2006. Vol. 167, pp. 665–681. 0003-0147/2006/16705-41049$15.00.

and survival, but the interactions among fitness correlates and their net effect on fitness remain unresolved. Resolution requires long-term data on survival, reproduction, and physiology, as well as robust estimates of fitness (Clark 1993; Cam et al. 2002).

Short-term studies are useful in evaluating physiological or behavioral mechanisms on fitness correlates (e.g., clutch size, parental care, nest success). However, when the life span of an organism exceeds the duration of the study or when selective agents change over the course of the study, synergistic interactions and cumulative effects of fitness correlates can remain undetected or can be interpreted incorrectly. For example, animals are known to compensate for decreased performance during one life stage by postponing the cost to a later life stage (Metcalfe and Monaghan 2001, 2003; Aihie Sayer and Cooper 2002; Ohlsson et al. 2002; Blount et al. 2003), which can compromise the interpretation of studies that focus on a single part of the life cycle. Birds, for example, are quickly able to compensate for periods of decreased nestling growth rates, but they pay a cost in lower survival and reproductive success much later as adults (Richner et al. 1989; de Kogel 1997; Ohlsson et al. 2002; Cam et al. 2003). Another weakness of short-term analyses of fitness is that changes in physiology and behavior may have effects on the fitness of other individuals in the population (e.g., other life stages or the opposite sex; Ketterson et al. 2001; J. M. Casto, V. Nolan Jr., and E. D. Ketterson, unpublished data), which are difficult to evaluate over the short term. The challenges of short-term studies are especially relevant for studies of hormone manipulations, where a single hormone can affect a large suite of phenotypic characters related to reproduction and survival. Although long-term measures of multiple fitness correlates are necessary to fully evaluate fitness costs, analysis of these data sets is complex.

We examine the consequences of reproductive endocrinology and behavior on fitness in a marked population of dark-eyed juncos (*Junco hyemalis carolinensis*), a songbird in which testosterone levels of adult males were experimentally manipulated over eight breeding seasons. We project changes in male fitness due to experimental testosterone treatment by considering its effects during all phases of the life cycle (egg, nestling, fledgling, 1-year-old adults, and 2-year-old and older adults). Testosterone levels were manipulated to mimic maximal levels observed in this population. The effects of testosterone treatment include changes in survival (estimated via mark-recapture methods), reproduction (e.g., clutch size, extra-pair fertilizations [EPFs]), and growth (e.g., body size, growth rate). We also evaluate the interaction between testosterone treatment and adult age (male and female) and its effect on reproductive performance and mate selection. We link the effects of testosterone treatment to fitness using population projection models that incorporate observed levels of variation in survival, reproduction, and growth among individuals.

Methods

Study Species and Area

We studied a population of dark-eyed juncos breeding in the Appalachian Mountains of Virginia from 1993 to 2001. Nolan et al. (2002) provide details on the life history and population trends of dark-eyed juncos in North America. The study site is heavily forested, with a canopy dominated by mixed deciduous hardwoods and conifers, and has been described in previous studies (e.g., Chandler et al. 1994). Adults in this population begin breeding in late April and early May. Males are territorial throughout the season, and pairs can rear as many as three broods per season (Ketterson et al. 1992, 1996). During the breeding season, adults are socially monogamous, and both parents care for young (Ketterson et al. 1992; Chandler et al. 1997); however, EPFs are common (Raouf et al. 1997; Ketterson et al. 1998). Juncos breeding in this area have been uniquely color marked and monitored as part of a long-term study of junco breeding biology since 1983 (Ketterson and Nolan 1999).

Population Monitoring

Each year, mist nets and Potter traps are used to capture dark-eyed junco adults and juveniles on the study site. Captures occur during two 3-week periods, one in early spring (late April through early May) and a second in late summer (late July through early August). At the time of initial capture, birds are banded with U.S. Fish and Wildlife Service (USFWS) aluminum bands and a unique combination of color bands. Measurements of body mass (pesola, 0.1 g), tarsus length (dial calipers, 0.1 mm), flattened wing and tail length, and plumage characteristics are recorded for all birds. Birds are sexed on the basis of plumage characteristics, wing length, and presence of brood patches (females) or cloacal protuberances (males). Birds are aged as juveniles (hatched in that breeding season), first-year (young, age 1), adults (hatched in the previous breeding season), or second-year or older (old, age 2+) adults on the basis of plumage characteristics and eye color (Hill et al. 1999). Many birds captured are of a known age as a result of banding efforts in previous years.

Nesting activity (building, laying, incubation, and brood rearing) is monitored for each pair nesting on the study site. Nests are visited daily during egg laying, every 3–6 days during incubation, and every 3 days during the nestling stage. During each visit at the nestling stage, nestling

mass (pesola, 0.1 g) and tarsus lengths (dial calipers, 0.1 mm) are measured. At 6 days of age, nestlings are banded with USFWS aluminum bands and a unique combination of color bands. A small blood sample is collected from the wing vein at this time for later determination of parentage. Nestlings fledge approximately 12 days after hatching and are cared for by both parents until gradually gaining independence approximately 3 weeks after fledging.

Testosterone Manipulation

From 1993 to 2000, testosterone levels were experimentally manipulated in breeding males in the study population. We inserted two 10-mm-long silastic tube implants subcutaneously (Dow Corning; 1.47 mm i.d., 1.96 mm o.d.) into nearly all males breeding at the study site and captured during early spring. Silastic tubes either contained crystalline testosterone (Sigma Chemical) to elevate circulating testosterone levels in one group of males (testosterone-treated males) or were left empty (control males). Implant methods are described in detail elsewhere (Ketterson et al. 1992). We checked implant status (presence or absence) when birds were recaptured within the season, and implants were removed from birds recaptured at the end of each breeding season. In subsequent years, recaptured males received the same implant treatment (testosterone or control) as in previous years. Males captured for the first time were blocked by age and capture site before being randomly assigned to either the testosterone or control treatment group and consistently received the same implant treatment in subsequent years. The population was monitored for returning individuals in 2001; however, no males received implants.

Model Description

We developed a three-stage postbreeding matrix population projection model to represent the dark-eyed junco life cycle. Model stages are juveniles, young adults (age 1), and old adults (age 2+), connected in the life cycle as shown in figure 1. We separated the adult stages into young and old adults because clutch size and nestling survival can be significantly lower for first-time breeders than for adults with previous breeding experience (Sæther 1990). This is an open population, and analyses of these age classes (juveniles, young adults, and old adults) assume that these are distinct groups (i.e., composed of different individuals). Although some individuals appear in multiple age classes, the vast majority of individuals appear in only one age class. In matrix form, the model is

$$\begin{bmatrix} \frac{C_1 A_1 S_1 \phi_F \phi_J}{2} & \frac{C_{2+} A_{2+} S_{2+} \phi_F \phi_1}{2} & \frac{C_{2+} A_{2+} S_{2+} \phi_F \phi_{2+}}{2} \\ \phi_J & 0 & 0 \\ 0 & \phi_1 & \phi_{2+} \end{bmatrix} \cdot \begin{bmatrix} N_J \\ N_1 \\ N_{2+} \end{bmatrix}_t = \begin{bmatrix} N_J \\ N_1 \\ N_{2+} \end{bmatrix}_{t+1}, \quad (1)$$

where C_1, A_1, and S_1 represent the clutch size, nesting attempts, and nest success of young adults, respectively; C_{2+}, A_{2+}, and S_{2+} represent the clutch size, nesting attempts, and nest success of old adults, respectively; ϕ_F is the survival of fledglings to the end of the summer; ϕ_J is the survival of juveniles from the end of the summer to the next spring; ϕ_1 and ϕ_{2+} represent annual survival of young and old adults, respectively; and N_J, N_1, and N_{2+} represent the number of juveniles, young adults, and old adults, respectively. Division by 2.0 occurs because only one sex is modeled to estimate fitness (i.e., rate of population change), and on the basis of data from this population, we assume that sex ratios are 50 : 50 (Grindstaff et al. 2001). The finite rate of change is set by the dominant eigenvalue (λ) of the system in equation (1) and also represents average fitness of the population, assuming density independence or equilibrium dynamics (Caswell 2001). Population parameters for the model were empirically derived from the observational data collected between 1993 and 2000 and are described below. Three primary groups are used for population modeling: testosterone-treated males, control males, and females. Demographic analyses were conducted to compare these groups and estimate population model parameters accordingly. Effects of age were also considered in the demographic analyses.

Estimating Population Parameters

We used a combination of statistical methods to estimate mean values and standard errors for the demographic parameters (reproductive and survival) used in the population projection models (JMP, ver. 5.0, SAS Institute 2002; program MARK, White and Burnham 1999). Statistical methods used to detect significant differences among demographic parameters included ANOVA and *t*-tests. We also used least squares regression, Monte Carlo simulation, and maximum likelihood methods to estimate parameters for the demographic models. Specific approaches used to test for differences among, and to estimate values for, each demographic parameter (clutch size, number of nesting attempts, nest success, EPFs, fledgling survival, juvenile survival, and adult survival) are detailed in the appendix in the online edition of the *American Naturalist*. In general, means and estimates of parameter variances were used to

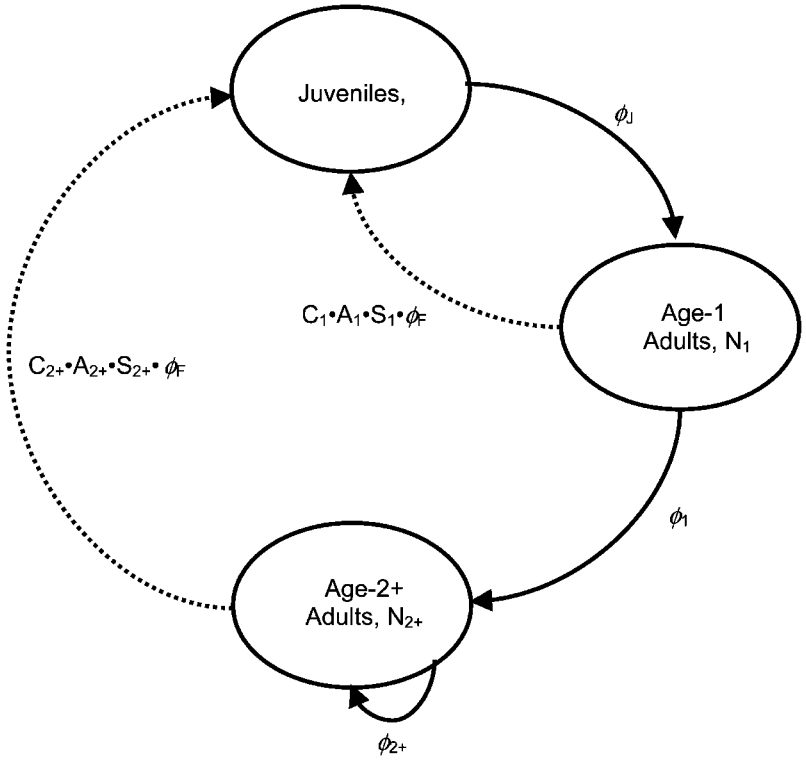

Figure 1: Dark-eyed junco life cycle used for the simulation model. Circles indicate distinct life stages, solid arrows indicate transition probabilities (i.e., survival from one life stage to the next), and dotted arrows indicate reproduction. Symbols used in the figure correspond to the demographic parameters from equation (1), where C_1, A_1, and S_1 represent the clutch size, nesting attempts, and nest success of young adults, respectively; C_{2+}, A_{2+}, and S_{2+} represent the clutch size, nesting attempts, and nest success of old adults, respectively; ϕ_F is the survival of fledglings to the end of the summer; ϕ_J is the survival of juveniles from the end of the summer to the next spring; ϕ_1 and ϕ_{2+} represent annual survival of young and old adults, respectively; and N_J, N_1, and N_{2+} represent the number of juveniles, young adults, and old adults, respectively.

estimate differences between male treatment and male and female age classes. These means and variance estimates were also included in the population projection models. In many cases, we included covariates (e.g., body size, age, year) to clarify relationships between life-history traits and fitness.

Nestling Growth

We also examined patterns in offspring growth to assess the effects of adult male testosterone treatment on offspring performance. We characterized nestling growth using a Janoschek growth curve (Gille et al. 1999) for measurements from 1,303 individual known-age nestlings that came from 442 different nests (219 nests belonging to control males and 223 belonging to testosterone-treated males). These models exhibit an S-shaped curve by characterizing size across nestling ages based on four independent parameters that reflect initial size (mass at hatching), asymptotic size (mass at fledging), a rate of growth parameter, and a parameter for the inflection point of the curve. We compared these measures of nestling growth between male treatment groups. Because small numbers of marked nestlings recaptured as juveniles or adults do not allow for direct analysis of the effect of male treatment or adult age on fledgling or juvenile survival rates, we also looked for effects of growth on fledgling survival by examining treatment and adult age effects on nestling mass at days 5–7 and at days 10–12 (see "Fledgling, Juvenile, and Adult Survival").

Differences in growth rates among treatment groups were evaluated using model selection criteria by calculating Akaike Information Criterion adjusted for small sample size (AIC_c) based on the error sums of squares resulting from least squares regression of Janoschek growth curves (see Burnham and Anderson 2002). In determining sample size for computing AIC_c values, we used the number of nests, not the number of nestlings in the two treatment groups, because individual nestlings within a nest are not independent. Candidate models were compared only after

a bootstrap goodness of fit (based on 200 replicates) on the most general (i.e., global) model in the set indicated that the fit was adequate (i.e., probability of observed deviance was <0.7; program MARK, White and Burnham 1999).

Model Simulations

We performed model simulations for five different scenarios to estimate the effects of hormone treatment on individual fitness. The five scenarios represent model demographic parameters based on testosterone-treated males acquiring no EPFs, control males acquiring no EPFs, testosterone-treated males acquiring EPFs, control males acquiring EPFs, and females. For each scenario, the simulation generated a distribution (and hence, mean and variance) for λ (i.e., mean fitness in the population; Caswell 2001). In all cases, we determined the distribution for λ from projections using the values of the demographic parameters estimated from simulations or empirical data and the life stage simulation analysis approach of Wisdom et al. (2000). For each scenario, we computed λ for 10,000 replicates in which matrix values (i.e., demographic parameters) differed from the mean value according to standard errors and random Gaussian deviates estimated from empirical data. A sine transformation based on mark-recapture modeling was applied first to deviates from the observed survival rates. From the 10,000 deviates, we then obtained the mean and distributional information on λ for each scenario. It is important to note that the simulated distributions cannot be used to make traditional statistical comparisons because the number of replicates (10,000) is arbitrary. We view the simulations as a method by which numerous components of the life history can be measured over the entire life span. Thus, components such as reproductive output and adult survival can be combined simultaneously to quantify their effects over many generations. By including the measured variability in the demographic parameters that make up the components of the projection model, we also account for demographic

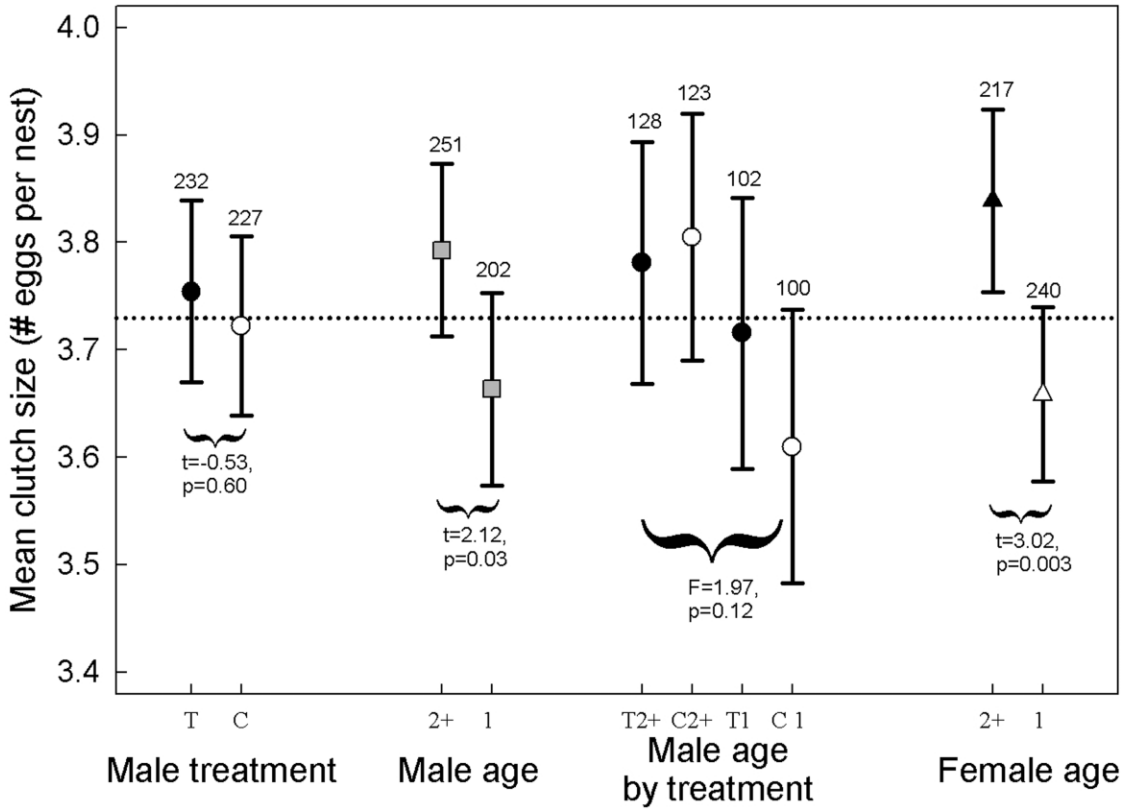

Figure 2: Mean clutch sizes by age, treatment, and sex for birds nesting from 1993 to 2001. Bars indicate 95% confidence intervals, and sample sizes are listed above the error bars. Filled and open symbols refer to testosterone-treated male and control male treatment groups, respectively; gray squares represent testosterone-treated male and control male groups combined; and triangles refer to females. ANOVA results indicate that young females and young males produce smaller clutches than old adults.

Table 1: Reproductive parameters for the Mountain Lake dark-eyed junco population

	Testosterone-treated males		Control males		Females	
Parameter	Young	Old	Young	Old	Young	Old
Clutch size[a]	3.72 ± .064	3.78 ± .057	3.61 ± .065	3.80 ± .058	3.66 ± .041	3.84 ± .043
	3.66 ± .045	3.79 ± .040	3.66 ± .045	3.79 ± .040		
Nest daily survival rate[b]	.940 ± .0037	.950 ± .0029	.940 ± .0037	.950 ± .0029	.945 ± .0063	.945 ± .0063
AIC_c w	.52				.85	
Duration (days)	73.14 ± 4.48	94.10 ± 4.00	78.35 ± 4.34	101.30 ± 4.47	81.26 ± 2.54	93.44 ± 3.02
Renest interval:						
Successful nest (days)	14.00 ± 2.19	12.67 ± 1.73	15.25 ± 2.03	14.80 ± 1.63	14.74 ± 1.39	13.66 ± 1.24
Unsuccessful nest (days)	9.00 ± 1.28	10.55 ± 1.06	9.54 ± 1.13	9.52 ± 1.00	10.23 ± .72	9.69 ± .79
Attempts	3.80 ± .72	4.34 ± .61	3.99 ± .75	4.71 ± .82	3.93 ± .62	4.54 ± .83
Fledgling survival:[c]						
Covariate:						
Mass days 10–12	.48 ± .04	.48 ± .04	.51 ± .04	.51 ± .04	.50 ± .06	.50 ± .06
AIC_c w	.48					
Mass days 5–7	.44 ± .03	.44 ± .03	.48 ± .04	.48 ± .04	.48 ± .07	.48 ± .07
AIC_c w	.30					
ΔAIC_c	.96					

Note: The upper rows for clutch size list mean and variance values used in the model simulations, while the lower rows indicate results from statistical analyses of the parameters (ANOVA, *t*-test). AIC_c w and ΔAIC_c indicate Akaike weight and difference in AIC_c value from the highest-ranked model (Burnham and Anderson 2002).

[a] For young and old testosterone-treated males, $F = 2.80$, df = 452, $P = .062$; $t = 3.017$, df = 456, $P = .0027$. For young and old females, $t = 2.12$, df = 452, $P = .0346$.

[b] Candidate models included effects from sex (S), male treatment (Tr), female age (FA), and male age (MA). The global model, $S_{S+Tr+FA+MA}$, had AIC_c $w = 0.02$, $\Delta AIC_c = 6.65$; a model with constant rate for all groups, S, had AIC_c $w = 0.15$, $\Delta AIC_c = 2.49$; $S_{S+FA+MA}$ had AIC_c $w = 0.08$, $\Delta AIC_c = 3.65$; S_{Tr+MA} had AIC_c $w = 0.08$, $\Delta AIC_c = 3.81$; S_{FA} had AIC_c $w = 0.08$, $\Delta AIC_c = 3.81$; S_{Tr} had AIC_c $w = 0.06$, $\Delta AIC_c = 4.20$; S_{Tr+FA} had AIC_c $w = 0.01$, $\Delta AIC_c = 7.52$.

[c] The global modal ϕ_F (constant survival, no covariate) had AIC_c $w = 0.22$, $\Delta AIC_c = 5.96$. Time period for the survival estimate is from fledging to the end of the birth summer.

variability among individuals in the population. Moreover, we conservatively estimated differences in demography among treatment (or age) groups by entering separate parametric values (and errors) into the models only when traditional statistical methods indicated a significant difference at the $P = .05$ level. Hence, the simulation results provide projected distributions of individual fitness values for the respective treatment populations with a conservative bias on differences.

Results

Reproduction

Adult age was the most consistent predictor of clutch size, number of nesting attempts, and nest success.

Clutch Size. Mean clutch sizes did not differ between male treatments but did differ by age class for both males and females (fig. 2). Old adults had larger clutch sizes than did young adults (old males: 3.79 ± 0.04 eggs; young males: 3.66 ± 0.05 eggs; $t = 2.12$, df = 452, $P = .0346$; old females: 3.84 ± 0.04 eggs; young females: 3.66 ± 0.04 eggs; $t = 3.017$, df = 456, $P = .0027$; table 1). An ANOVA in which all factors (male treatment, male age, female treatment, and all interaction terms) were included indicated that differences in clutch size were affected by female age, whereas male age effects were an indirect effect of female age (older males being more likely to mate with older females; see "Mate Selection"). Note that despite the indirect nature of the effect of male age, we nonetheless included separate estimates for old and young males when entering estimates for matrix parameters for projecting male fitness because assortative mating could not be directly included in the projection model because of limitations of simultaneously assessing fitness of both males and females.

Number of Nesting Attempts. There was no detectable difference in average duration of the breeding season (computed from the number of equally good weeks with bootstrap-calculated standard deviations) between male treatment groups (i.e., means are within 2 SD of one another), but there were detectable differences between age classes for both males and females. Older adults had longer season durations (values from all simulations of nesting

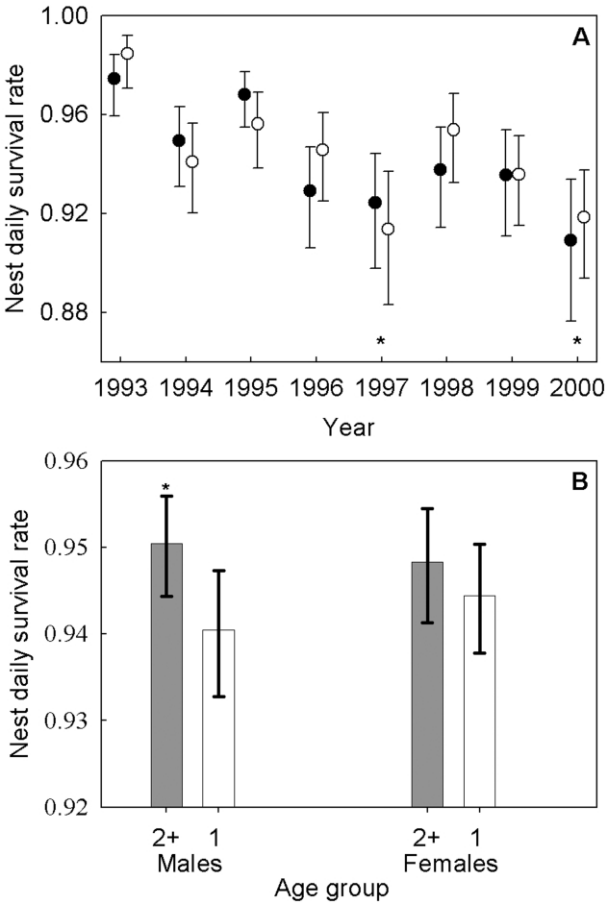

Figure 3: Daily survival rates of nests from 1993 to 2000 by (*A*) year and male treatment group (*filled circles* = testosterone-treated males; *open circles* = control males) and (*B*) male and female age groups (*shaded bars* = old adults; *unshaded bars* = young adults). Error bars indicate 95% confidence intervals. ΔAIC_c values indicate that rates differ by year (*A*) and that older males have higher nest success than young males (*B*).

attempts are means ±1 SD; testosterone-treated males: 94.1 ± 4.0 days; control males: 101.3 ± 4.5 days; females: 93.4 ± 3.0 days) than young adults (testosterone-treated males: 73.1 ± 4.5 days; control males: 78.4 ± 4.3 days; females: 81.3 ± 2.5 days; table 1). The simulations of season-long numbers of nesting attempts showed that old adults (males and females) are able to make more nesting attempts per breeding season than young adults. The simulated number of nesting attempts for old males (testosterone-treated males: 4.34 ± 0.61; control males: 4.71 ± 0.82) was higher than the number of attempts for young males (testosterone-treated males: 3.80 ± 0.72; control males: 3.99 ± 0.75). Similarly, the number of nesting attempts for old females (4.54 ± 0.83) was higher than the number of nesting attempts for young females (3.93 ± 0.62). The differences in simulated number of attempts between age groups arose from an age difference in nest success, which was greater in old males than in young males, as well as an age difference in duration of the breeding season, which was also greater in old adults. The distributions observed for the respective age and treatment groups were entered into the population projection simulations.

Nest Success. Nest success did not differ between male treatments but did differ across years of the study and male age groups (table 1; fig. 3*A*). Unlike males, female age class did not affect nest success. Average daily nest survival rates generally declined over the study period from a high of 0.9846 in 1993 to a low of 0.9092 in 2000 (fig. 3*A*). According to the most parsimonious statistical model selected using AIC_c, nest success estimates during years 1997 and 2000 were detectably lower than during other years but were not detectably different from one another (fig. 3*A*). Analyses in which nests were pooled across all years indicated that older males had detectably higher daily nest survival than young males (old males: 0.950 ± 0.0029 daily nest survival rate; young males: 0.940 ± 0.0037 daily nest survival rate; AIC_c weight (w) = 0.519, $\Delta AIC_c = 0$; table 1; fig. 3*B*).

EPFs

Approximately 80% of the nestlings used in the analyses of EPFs were correctly assigned to male parents in the study population. The remaining 20% were unassigned to male parents, which indicates that these nestlings were fathered by males that were not monitored from this breeding population (either offsite or not captured during the study period). For the simulation models, we assume that the correctly assigned nestlings accurately represent the proportion of all nestlings in both testosterone-treated and control male nests.

EPF Losses. EPF losses varied significantly among years ($F = 4.969$, $P = .021$); however, in all years combined, male treatment and male age had no significant effect on EPF losses (treatment: $F = 0.037$, $P = .847$; age: $F = 1.151$, $P = .285$), and there was no significant interaction between treatment and age ($F = 0.297$, $P = .587$; fig. 4).

EPF Gains. EPF gains varied significantly among years ($F = 2.575$, $P = .021$). In all years combined, both male age and male treatment had significant effects on gains (age: $F = 4.087$, $P = .045$; treatment: $F = 3.994$, $P = .048$), but no interaction between treatment and age was detected ($P = .303$; fig. 4).

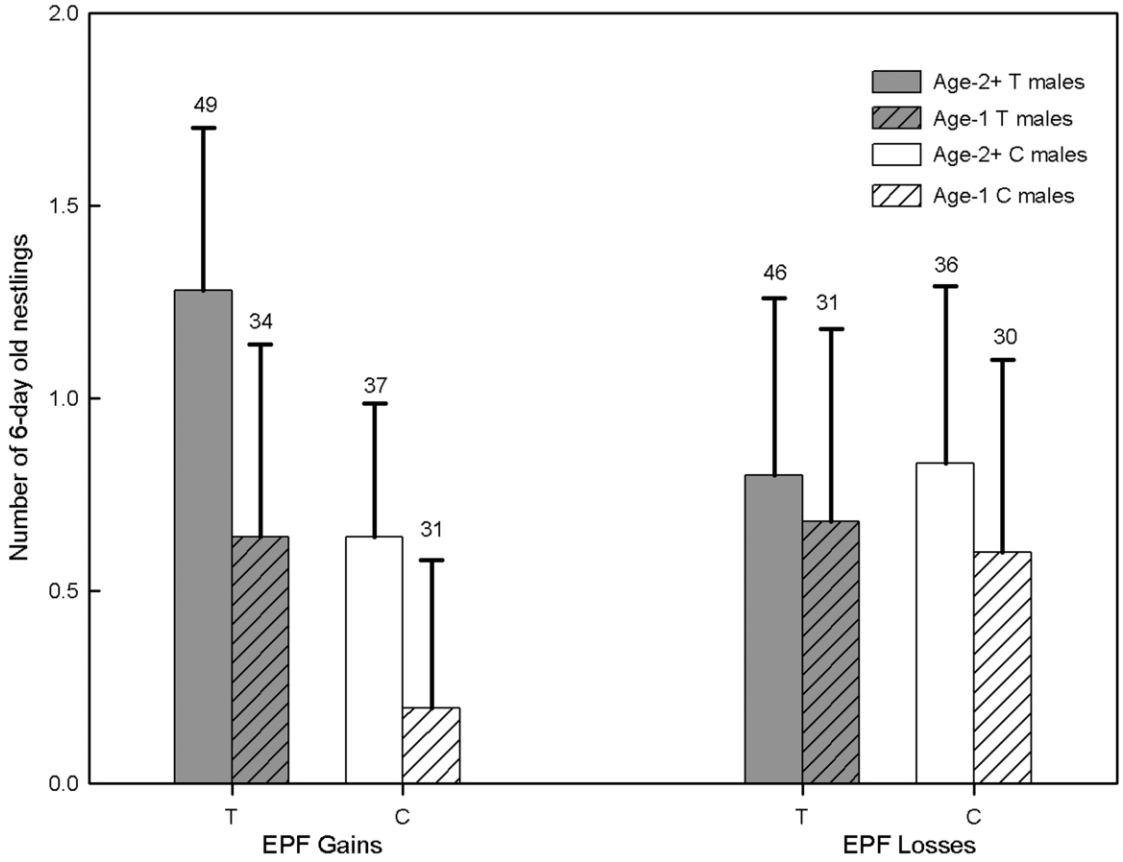

Figure 4: Mean extra-pair fertilization (EPF) gains and losses by male age and treatment from 1990 to 1996. Numbers above the 95% confidence limit bars indicate the number of day 6 nestlings in each category. Male age and treatment did not explain EPF losses. EPF gains vary depending on male age and treatment. Old males have higher EPF gains than young males, and testosterone-treated males have higher gains than control males.

Fledgling, Juvenile, and Adult Survival

Fledgling survival rates (from nest leaving to the end of the summer) did not differ between male treatments. Mark-recapture analysis, with results from survival analysis using program MARK, indicated that nestling mass at the midpoint of nestling life (days 5–7) and nestling mass at fledging (days 10–12) affected fledgling survival rates (mass days 5–7: AIC_c $w = 0.30$, $\Delta AIC_c = 0.96$; mass days 10–12: AIC_c $w = 0.48$, $\Delta AIC_c = 0.0$; table 1; fig. 5*A*).

Juvenile survival rates did not differ between male treatment groups. Most juveniles are captured in late summer mist netting efforts, and the nest of origin (and therefore male treatment group) is unknown. The low numbers of juveniles whose male parents were known precluded our ability to develop a survival model using only juveniles from testosterone-treated and control males with differences between treatment groups that had adequate goodness of fit. However, we obtained an estimate for juvenile survival by including encounter histories from all juveniles (those from testosterone-treated males and control males as well as those of unknown origin) and modeled survival as constant (0.53 ± 0.02; table 2).

Results of mark-recapture analysis indicated that testosterone-treated males had lower apparent annual survival than control males. The most parsimonious statistical model (based on AIC_c) from program MARK included different apparent annual survival rates for testosterone-treated male and control male groups but no differences in capture probability (table 2; fig. 5*A*). The apparent survival rate for testosterone-treated males was lower than the rate for control males (0.38 ± 0.03 vs. 0.44 ± 0.03, respectively; AIC_c $w = 0.37$, $\Delta AIC_c = 0.0$; table 2; fig. 5*A*), and these survival values and capture probabilities did not vary with year. We did not detect age effects on

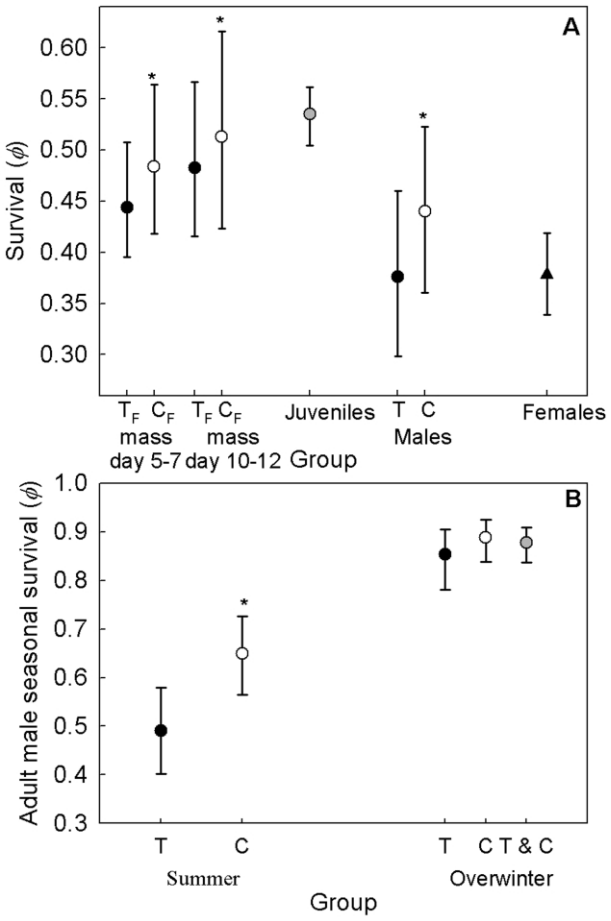

Figure 5: *A*, Survival of dark-eyed juncos observed from 1993 to 2000 by life stage and treatment groups. *B*, Seasonal survival of adult males by treatment group. Fledgling survival rates for nestlings reared by testosterone-treated and control males (T_F and C_F, respectively) are shown according to mean nestling mass at days 5–7 and days 10–12. Error bars indicate 95% confidence intervals; filled and open circles refer to testosterone-treated males and control males, respectively; and gray circles are for combined groups. ΔAIC_c values indicate that annual male survival differed by treatment (*A*; testosterone-treated males vs. control males) and that the difference in survival occurred during the summer rates (*B*).

either survival or capture probability. Adequate goodness of fit was confirmed on the global model using the bootstrap procedure described previously.

Furthermore, mark-recapture results indicate that apparent survival within the breeding season was detectably lower in testosterone-treated males than in control males, but there was no difference in overwinter survival between male treatment groups. The most parsimonious model indicated that apparent survival within the breeding season (3-month period) was 0.49 ± 0.04 for testosterone-treated males compared with 0.65 ± 0.04 for control males, but overwinter survival did not differ between groups (0.88 ± 0.02 per 3-month period, or approximately 0.68 survival for the entire 9-month period; AIC_c $w = 0.34$, $\Delta AIC_c = 0.0$ vs. AIC_c $w = 0.0081$, $\Delta AIC_c = 7.5$ for a model with no difference in survival; fig. 5*B*). Adequate goodness of fit was confirmed on the global model using the previously described bootstrap procedure.

Results of mark-recapture analysis for female juncos indicate that annual survival did not vary with year or age. The most parsimonious statistical model had constant apparent annual survival and capture probabilities (AIC_c $w = 0.65$, $\Delta AIC_c = 0.0$; table 2; fig. 5*A*). Likewise, the most parsimonious statistical model for juveniles reared by testosterone-treated and control males (juvenile survival is from late summer to the following spring) had constant survival and capture probabilities (AIC_c $w = 1.0$, $\Delta AIC_c = 0.0$; table 2; fig. 5*A*).

Nestling Growth

Results indicate that male treatment affected nestling growth. Analysis of the Janoschek growth model fit to measurements from 1,303 nestlings from 442 nests indicates that the most parsimonious model has a lower parameter for the asymptote of nestlings from testosterone-treated males than for nestlings from control males (asymptotic mass = 16.75 and 17.14 g, respectively; $AIC_c = 1{,}217.84$, $\Delta AIC_c = 0$; fig. 6). A null model with no differences in growth curve parameters between treatment groups had $\Delta AIC_c = 1.50$, and a global model in which all parameters were different had $\Delta AIC_c = 1.96$.

Mate Selection

Pairings of males and females were not independent (i.e., random) with respect to age or treatment. Older females were more likely to pair with older males than with young males (likelihood ratio test with $\chi^2 = 20.36$, df = 1, $P = .0001$; $\chi^2 = 12.293$, df = 1, $P = .0005$; fig. 7). However, young testosterone-treated males were more likely than young control males to mate with older females ($\chi^2 = 7.06$, df = 1, $P = .0079$; $\chi^2 = 4.62$, df = 1, $P = .032$; $\chi^2 = 19.83$, df = 3, $P = .0002$; fig. 7).

Simulation Results

The cumulative effect of all survival and reproductive parameters using the simulation model indicated that fitness differs between male treatment groups. Results from the simulation model showed that λ was lower for testosterone-treated males than for control males (mean ± SD 0.93 ± 0.108 vs. 1.08 ± 0.110, respectively; fig. 8*A*). However, when EPFs were considered, testosterone-treated males realized a detectably higher λ than control males

Table 2: Survival estimates from the most parsimonious models for the Mountain Lake dark-eyed junco population

	Testosterone-treated males		Control males		Females	
Parameter	Young	Old	Young	Old	Young	Old
Juvenile survival[a]	.53 ± .02	.53 ± .02	.53 ± .02	.53 ± .02	.53 ± .02	.53 ± .02
AIC_c w[b]	1.0					
Adult annual survival[c]	.38 ± .03	.38 ± .03	.44 ± .03	.44 ± .03	.38 ± .03	.38 ± .03
AIC_c w	.37				.65	

[a] Time period for survival estimate is from the end of the birth summer to the beginning of the following breeding season.

[b] Global model.

[c] Candidate models included effects of male treatment (Tr) on apparent survival (ϕ) and recapture probability (p) for males and female age (FA) for females. The global model for males, ϕ_{Tr}, p_{Tr}, had AIC_c $w = 0.32$, $\Delta AIC_c = 0.29$; ϕ, p had AIC_c $w = 0.22$, $\Delta AIC_c = 1.07$; ϕ, p_{Tr} had AIC_c $w = 0.09$, $\Delta AIC_c = 2.62$. The global model for females, ϕ_{FA}, p_{FA}, had AIC_c $w = 0.11$, $\Delta AIC_c = 3.59$; ϕ_{FA}, p, had AIC_c $w = 0.24$, $\Delta AIC_c = 1.92$.

(mean ± SD 1.28 ± 0.157 vs. 1.08 ± 0.120; fig. 8*B*). Although we cannot evaluate the statistical significance of the differences in the fitness distributions (see "Methods"), the degree of the difference (approximately 35% between testosterone-treated males and control males when EPFs are included) indicates that a measurable change in dynamics occurs with the inclusion of EPFs in the model structure (see Murdoch et al. 1992 for further information). The mean and standard deviation of λ calculated for females was 1.00 ± 0.092, which indicated approximately stable population growth.

Discussion

On the basis of long-term field data and population projections, we found that experimentally elevated testosterone resulted in decreased apparent survival for males during the breeding season but not during the winter after treatment had ended. Treatment with testosterone had no detectable effect on nest success, but it increased male reproductive success via EPFs. Increased EPFs outweighed decreased survival in terms of net impact on fitness, resulting in higher overall fitness in testosterone-treated males. Treatment with testosterone also had extended phenotypic effects on offspring and females. Nestlings of testosterone-treated males weighed less than nestlings of control males, and smaller nestlings were less likely to survive the period between leaving the nest and late summer; however, treatment of the male parent had no detectable direct effect on offspring survival during the first winter of life. Treatment of males with testosterone affected females by increasing the likelihood that older females would mate with young testosterone-treated males. Because young males treated with testosterone have lower nest success than older males and also produce smaller nestlings (which indirectly indicates lower fledgling survival rates) than control males, females paired with young testosterone-treated males may realize reduced reproductive success.

Adult Survival

The decreased apparent survival of testosterone-treated males during the summer breeding season suggests a tight link between mortality and the effects of testosterone on breeding season behaviors and physiology. Relative to control males, testosterone-treated males increase activity levels and home range sizes (Chandler et al. 1994, 1997), increase food consumption rates (Lynn et al. 2000; Clotfelter et al. 2001), feed young at a lower rate (Ketterson et al. 1992), and have elevated levels of stress hormones (Ketterson et al. 1991; Klukowski et al. 1997; Schoech et al. 1999; McGlothlin et al. 2004) and decreased immune function (Casto et al. 2001). In some situations, these traits can be beneficial to males, but our results indicate that the net effect is to reduce life expectancy (survival). For example, increased activity levels of testosterone-treated males and higher levels of song and courtship (Enstrom et al. 1997) may increase the ability of a male to attract females but may also increase detectability and susceptibility to predation. Likewise, stress hormones play an important role in mobilizing energy in the face of immediate threats; however, chronic elevation of stress hormones can result in decreased immune function, altered metabolic pathways, and reduced fat reserves (Silverin 1986; Folstad et al. 1989; Wingfield 1994), all of which are consistent with increased susceptibility to disease. Results of analyses presented here clearly indicate that survival of testosterone-treated males is lower than survival of control males and that these costs are incurred during the breeding season (fig. 5). Without significant treatment-related differences in other demographic parameters, the prediction is that males with naturally prolonged and elevated levels of testosterone would have reduced fitness and that this phenotype should not persist in the population.

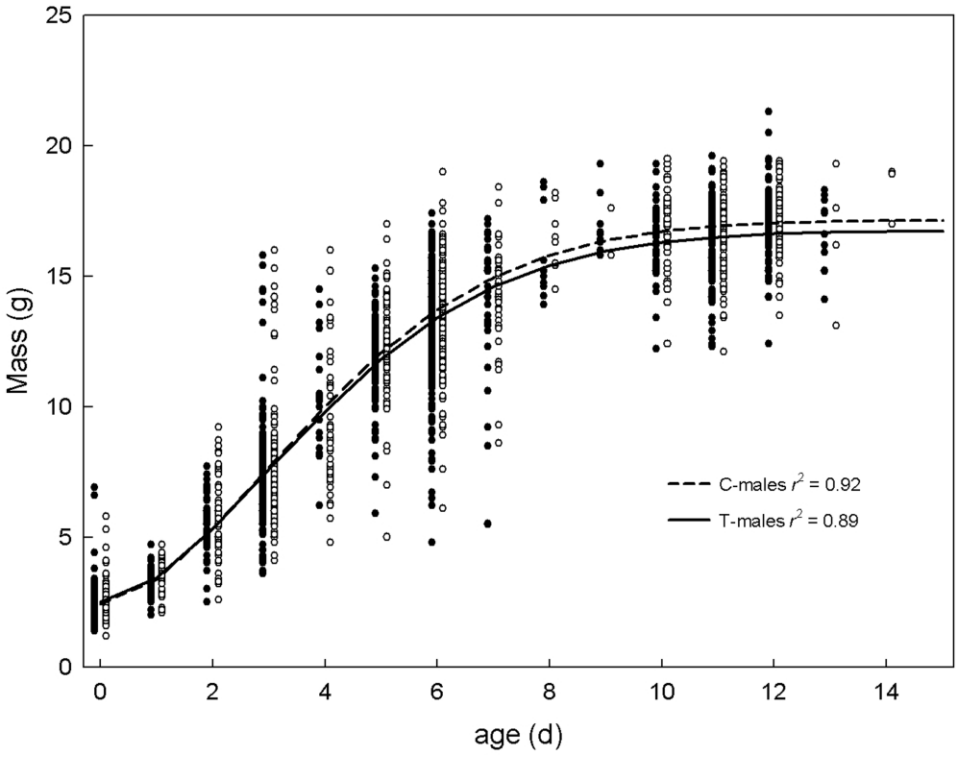

Figure 6: Growth of dark-eyed junco nestlings from 1993 to 2000 given by mass at age over the nesting period separated by treatment. Filled circles represent nestlings reared by testosterone-treated males, and open circles represent nestlings reared by control males. The most parsimonious growth model (determined by AIC_c values), in which the mass at fledging is higher for nestlings reared by control males (*dashed line*) than for nestlings reared by testosterone-treated males (*solid line*), is shown.

However, adult survival is only one component of an individual's fitness. Reproductive success can compensate for decreased survival and can substantially affect fitness (Schuster and Wade 2002), and because testosterone has been shown to increase male attractiveness to females (Enstrom et al. 1997; Hill et al. 1999) and decrease parental behavior (Ketterson et al. 1992; Schoech et al. 1998), we anticipated differences in reproductive success. We found relatively few differences in reproductive performance between testosterone-treated and control males, but those we did find are key in understanding male fitness. On the basis of data from 7 years, as opposed to data previously reported from 4 years (table 3; fig. 4), we confirmed an earlier report of greater incidence of gains in reproductive success through EPFs by testosterone-treated males (Raouf et al. 1997). Higher EPFs likely arise from changes in behaviors mediated by testosterone, probably most importantly the increase in home range size, courtship behaviors, and attractiveness associated with testosterone (Chandler et al. 1994; Enstrom et al. 1997; for discussion, see Raouf et al. 1997). The ability of testosterone-treated males to gain higher genetic reproductive success through EPFs compensates for lower survival and ultimately results in higher fitness for testosterone-treated males compared with control males.

Relation to Evolution of Mating Systems and Life Histories

In this study, testosterone levels were manipulated to mimic the maximum levels detected in unmanipulated birds and to maintain these levels over the entire breeding season. The pattern of manipulated testosterone levels differs from the short rise, peak, and fall in testosterone that is typical of juncos and other socially monogamous songbirds of the temperate zone in which males provide significant parental care. Prolonged elevation in testosterone is instead typical of species with polygynous mating systems in which little to no male parental care is provided (Wingfield et al. 1990). The concordance between pattern of testosterone secretion and life history suggests that natural selection acts on hormone-mediated behaviors to produce variation in avian breeding, mating, and parental care strategies. In the case of juncos, the testosterone-treated males were a "deviant" phenotype and as such would be

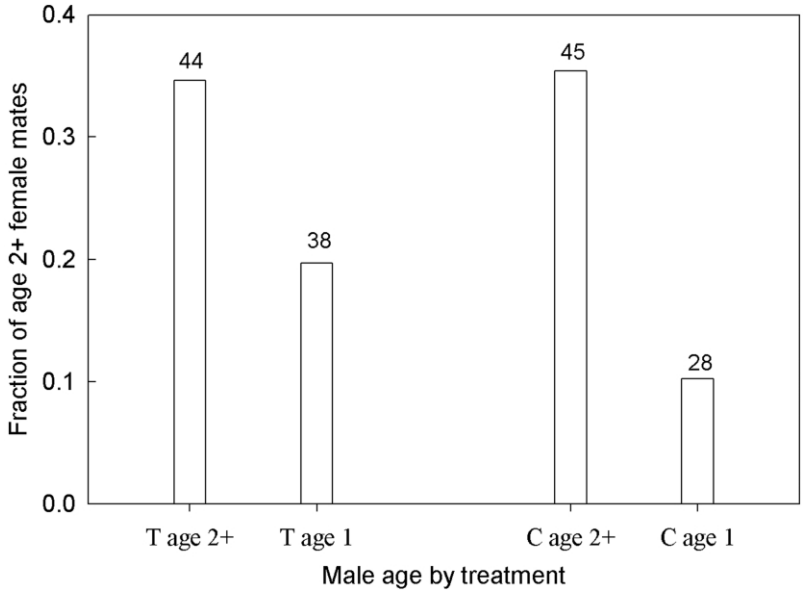

Figure 7: Mate pairings of age 2+ females by male age and treatment groups from 1993 to 2000. Numbers above the bars refer to total pairs of each category observed. A contingency analysis indicates that young males treated with testosterone are more successful in attracting older females than young control males.

expected to have lower fitness (as discussed in Ketterson et al. 1992; Ketterson and Nolan 1999). However, as shown here, their fitness achieved through EPFs actually exceeds that of the typical phenotype (control male). Given the ability of juncos to achieve higher testosterone levels naturally, the fitness differences we observed would predict directional selection resulting in elevated testosterone in males, with the strength of this selection reflected in the differences in mean fitness observed between testosterone-treated and control males in figure 8*B*.

Several considerations may resolve this apparent paradox in relative abundance of male phenotypes. One explanation is related to the dependence of the high-testosterone phenotype on males with the normal-testosterone phenotype. If the frequency of the high-testosterone phenotype increases in the population, the EPF losses among these males would also increase as the number of males with more typical testosterone phenotype decrease. Thus, the benefits of EPFs to high-testosterone males are greatest when this phenotype is relatively rare in the population because as the high-testosterone phenotype increases, these males would eventually pay a cost in fitness (i.e., EPF losses to other high-testosterone males). We are unable to fully explore the consequences of frequency dependence of these phenotypes with the data currently available, but such a dynamic modeling approach would be interesting. Several lines of evidence from our results indicate that if males with the phenotype of the testosterone-treated males were to arise, this phenotype may not spread, owing to subsequent consequences to females mated to these males. One line of evidence is the extended effect of male testosterone level on the phenotype and fitness of offspring as well as the fitness of females. Poor parenting by testosterone-treated males is expected to indirectly decrease offspring fitness. A second line of evidence is the effect of elevated testosterone on the behavior of young males and female mate choice. Over time, females would be expected to decrease their preferences for phenotypes mediated by testosterone. We used a single-sex model to assess fitness because of limitations in the current observational and field studies (the study was focused on male reproductive phenotypes). Strong selection against females choosing males with high levels of testosterone may ultimately constrain elevated testosterone levels in males.

Extended Effects of Testosterone Treatment on Offspring and Females

In the first case, elevated levels of testosterone in breeding males are known to decrease male contributions toward parental care in a variety of species (Silverin 1980; Hegner and Wingfield 1987; Saino and Møller 1995; Ketterson et al. 1996; but see Lynn et al. 2005), including juncos (Ketterson et al. 1992; Schoech et al. 1998). In this study, we found an effect of treatment on nestling growth rates (fig.

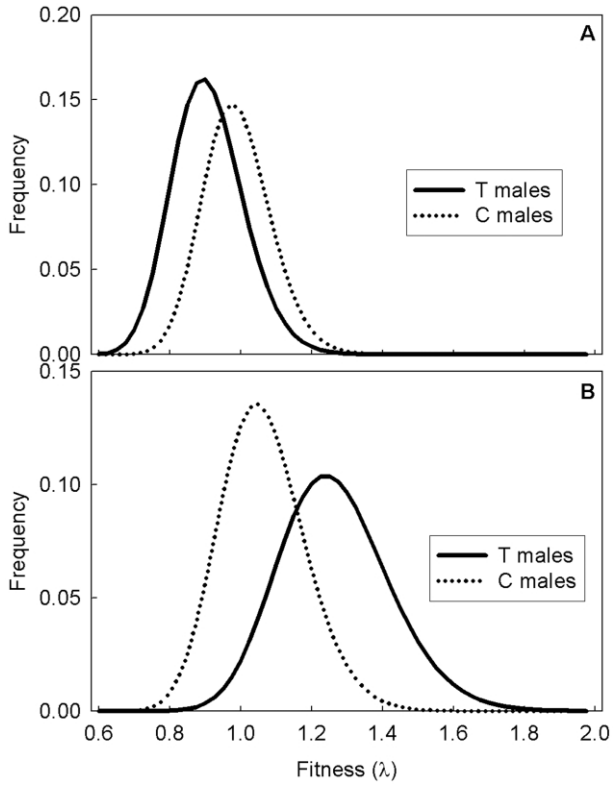

Figure 8: Distribution of fitness predicted by the simulation model for testosterone-treated males (*solid line*) and control males (*dashed line*) when (*A*) extra-pair fertilizations (EPFs) are ignored and (*B*) EPFs are included

6). Nestlings from testosterone-treated male nests fledge at smaller sizes than nestlings from control male nests (fig. 5*A*), which corroborates previous findings of decreased parental care (e.g., lower nest visitation rates and lower feeding rates) among testosterone-treated males in this population (Ketterson et al. 1992; Schoech et al. 1998). Reductions in nestling growth rates have direct consequences for reproductive success because survival as a fledgling is positively correlated with nestling body mass (fig. 6).

In addition to the direct effects on survival, conditions during early development are known to have long-term effects on performance as adults (Aihie Sayer and Cooper 2002). For example, in the kittiwake, the length of the nestling period and the rank of siblings within nests, both measures of parental provisioning, are positively correlated with survival to recruitment into the breeding population, age at recruitment, and reproductive performance as adults (Cam et al. 2003). These persistent effects of conditions during early growth and development on future survival and reproduction can have large impacts on population and evolutionary dynamics. In organisms that breed seasonally, poor environmental conditions during the time young are being raised may result in an entire cohort that has reduced survival and reproductive performance as they enter the breeding population. Similarly, heterogeneity in adult efficacy when raising young could be a mechanism responsible for the heterogeneity in breeding performance of individuals as breeding adults. In the case of the dark-eyed juncos, males treated with testosterone raise young that are smaller than young raised by control males, which could effect future reproductive success as adults.

Male juncos are able to significantly enhance fitness through the production of extra-pair young; however, female juncos do not use an analogous strategy (i.e., conspecific brood parasitism). Because of this, female fitness is more intimately tied to the number and quality of young raised in their own nests, and decreased offspring quality and survival of young when paired with testosterone-treated males likely has a larger impact on their fitness relative to male fitness. Additionally, female juncos are known to compensate for reductions in male parental care (Wolf et al. 1990, 1991; Ketterson and Nolan 1992; Ketterson et al. 1992), which may directly decrease female fitness via reductions in her survival and future reproductive potential (Wolf et al. 1991). Testosterone-treated males make fewer nest visits during the nestling period, resulting in less food delivered and less time spent at the nest than control males (Ketterson et al. 1992; Schoech et al. 1998). These males also have a delayed response to predator threats compared with control males (Ketterson et al. 1996; Cawthorn et al. 1998). Females mated to testosterone-treated males appear to be able to compensate for the reduction in parental care; however, compensation may come at a cost to a female's own condition. When males are prevented from participating in parental care duties and females raise nestlings exclusively, females suffer greater rates of mass loss over the season relative to females that are aided by males (Wolf et al. 1991; Casto et al. 2001). Furthermore, females mated to testosterone-treated males have a lowered immune function relative to females mated to control males (Ketterson et al. 2001). At this time, we are unable to evaluate directly the fitness consequences for females mated to testosterone-treated versus control males. The focus of this study has been the direct effect of testosterone on male physiology, behaviors, and demography. Although we are able to assess correlates of fitness for females mated to males in each treatment over short time periods, females did not consistently mate with males in a particular treatment, and we are unable to evaluate the overall fitness consequences of mate treatment for females.

Table 3: Empirical estimates of the number of extra-pair fertilizations (EPFs) per day 6 nestling for young and old males in each treatment group

	Testosterone-treated males		Control males	
Parameter	Young	Old	Young	Old
EPFs per day 6 nestling[a]	.156 ± .079	.390 ± .069	.029 ± .085	.151 ± .075
Adjusted clutch size	3.23 ± .13	3.29 ± .13	2.92 ± .12	3.07 ± .12

Note: The adjusted clutch size refers to the clutch sizes adjusted for EPFs as estimated from the Monte Carlo simulations and used in the model simulations.

[a] $F = 4.14$, $P = .0074$.

Effects of Testosterone Treatment on Young Males

A second way that a male phenotype with elevated testosterone might be prevented from spreading is through signals that affect mate choice. Elevated levels of testosterone during the breeding season are synergistic with the effects of age. Old females prefer to mate with old males (fig. 7), and these pairings result in the highest values of reproductive performance (figs. 2, 3). Older birds breed for a longer period during the season, produce larger clutches, and are more likely to have successful nests than are young birds. The positive relationship between age and reproductive performance is well established in birds (Sæther 1990; Forslund and Part 1995; Cam and Monnat 2000; Wiktander et al. 2001). However, the specific factors responsible for improved reproductive performance with increased age are not resolved.

Older female juncos clearly preferred to mate with older males; however, elevated testosterone levels in young males significantly increased their likelihood of pairing with an old female (fig. 7). Mating with a young male negatively impacts reproductive success from the female perspective because young birds have lower values of reproductive performance (figs. 2, 3). Typically, young male juncos court and sing less vigorously and have lighter plumage than old males (Nolan et al. 2002) and also have lower circulating levels of testosterone (Ketterson and Nolan 1992; Deviche et al. 2000). Many of the behavioral differences between young and old males are sensitive to testosterone levels (Ketterson and Nolan 1992). For example, treatment with testosterone increases young male song rates, activity levels, and courtship behaviors (Enstrom et al. 1997) and causes young males to be as attractive to captive females as older males (Nolan et al. 2002). Our results suggest that when testosterone levels are elevated in young males, behavioral differences between the ages diminish and increase the likelihood of older females pairing with young males. Although young testosterone-treated males act like old males, they do not realize the same reproductive performance as old males (figs. 2, 3). Young males have decreased nest success, clutch sizes, and season durations compared with older males. Hence, this suggests that male experience (and not just physiological maturity) contributes to reproductive success and that females use testosterone-mediated physiological and behavioral cues to assess the experience of potential mates and select mates that offer the highest likelihood of reproductive success based on that experience. Elevated testosterone levels in males might ultimately be constrained by refinements in the cues females use to select mates.

Two competing theories on the evolution of female mate selection suggest either that females use cues to select mates that reflect good genes that will be passed on to their offspring (Hamilton and Zuk 1982; Folstad and Karter 1992) or that females use arbitrary cues that do not necessarily reflect male fitness (for discussion, see Schuster and Wade 2002). Our results suggest that females use testosterone-mediated traits to assess age of their mates, which may be an arbitrary trait with respect to genetic quality but an important trait with respect to a male's contribution to reproductive performance (nest success and nestling rearing). Although testosterone-treated males achieve higher fitness by increasing mating opportunities, the evolution of higher testosterone levels in males may be constrained if there is sexual selection (mate choice) for testosterone-mediated traits only when they are an honest signal of age and breeding experience.

Conclusion

Hormone manipulation studies have provided insight into physiological and behavioral mechanisms underlying life-history trade-offs (Ketterson et al. 1992; Stearns 1989; Ketterson and Nolan 1999; Zera and Harshman 2001). We combine this experimental approach with population projection models to evaluate these physiological and behavioral mechanisms in the appropriate context for fitness: cumulative effects of these mechanisms over the complete life cycle. Our study provides new insight into developing frameworks for dynamic models that are more appropriate for understanding life-history evolution than multiple analyses based on single correlates of fitness (Lande 1982). We incorporated observed levels of individual variation in multiple life-history traits affected by male testosterone

levels to evaluate potential trade-offs among survival, reproduction, and maturation as constraints on the evolution of reproductive physiology in dark-eyed juncos.

Acknowledgments

Many thanks to the numerous collaborators, postdoctoral associates, and graduate students who followed thousands of juncos breeding between 1993 and 2000: C. Aron, D. Aylor, J. Casto, E. Clotfelter, B. Garrison, J. Grindstaff, E. Hagen, M. Hill, D. Hopenstand, J. Kleffner, J. McGlothlin, I. Parker-Renga, K. Schubert, and D. Ziolkowski. We thank H. Wilbur and the Mountain Lake Biological Station of the University of Virginia for providing facilities and support during the field seasons and the Mountain Lake Hotel and Wilderness Conservancy for providing access to the study area. This article was significantly improved through helpful comments and discussions from E. Cam, A. Zera, and an anonymous reviewer. This work was supported with funds from National Science Foundation grants BSR 91-11498, BSR 87-18358, IBN 94-08061, and IBN 97-28384 to E.D.K. and V.N.

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Associate Editor: Ben C. Sheldon
Editor: Jonathan B. Losos

Junco hyemalis, dark-eyed junco adult (photograph by Will Clark).