

# High Testosterone Prior to Song Crystallization Inhibits Singing Behavior in Captive Yearling Dark-Eyed Juncos (*Junco hyemalis*)

Russell C. Titus,<sup>1</sup> Ellen D. Ketterson, and Val Nolan Jr.

Department of Biology, Indiana University, Jordan Hall, Bloomington, Indiana 47405

Received December 23, 1996; revised April 23, 1997; accepted May 19, 1997

In passerine birds, song is considered crucial in advertising reproductive and territorial status to conspecifics. Variation in the quality and frequency of song may be influenced by hormonal effects during the individual's development. This variation in turn may affect the function and potency of song. We studied the influence of testosterone on vocal production in first-year male dark-eyed juncos (*Junco hyemalis*), using subcutaneous silastic implants filled with testosterone. Subjects were visually but not acoustically isolated from one another and after capture had no exposure to female or adult male models. Implants were administered when subjects were in the plastic song phase (i.e., after they had begun to sing but before song was fully crystallized). Control males (C males) received empty implants. Experimental males were of two classes: TI males received one dose of testosterone (a single 10-mm implant), and TII males received two doses. Testosterone implants kept plasma levels high well into the breeding season, whereas in nature, levels normally drop after territorial acquisition and pair formation. Control males sang at higher rates than testosterone-treated males of both classes and had the greatest number of song types. This inhibitory effect of testosterone on vocal production suggests that disturbance of seasonal profiles of testosterone in birds may interfere with the production of species-typical song. © 1997 Academic Press

The steroid hormone testosterone is involved in the development and expression of male reproductive behavior in many avian species. To explore the costs and benefits of testosterone with respect to reproduction and survival in free-living dark-eyed juncos (*Junco hyemalis*), Ketterson, Nolan, and colleagues have experi-

mentally elevated and maintained testosterone levels and measured ensuing effects on behavior and physiology (Ketterson, Nolan, Wolf, Ziegenfus, Dufty, Ball, and Johnsen, 1991a; Ketterson, Nolan, Wolf, and Ziegenfus, 1992; Ketterson and Nolan, 1992; Ketterson, Nolan, Cawthorn, Parker, and Ziegenfus, 1996). Treatment with testosterone produces numerous behavioral changes, including increased song production as measured both in the vicinity of the male's nest and throughout his home range (Ketterson *et al.*, 1992; Chandler, Ketterson, Nolan, and Ziegenfus, 1994).

Experimental raising of testosterone levels increases vocal production in a wide variety of avian species in addition to juncos (Silverin, 1980; Balthazart, 1983; Hegner and Wingfield, 1987). At a finer level of analysis, testosterone has been associated with changes in the acoustical structure of vocalizations, including stereotypy of songs (Nottebohm, Nottebohm, Crane, and Wingfield, 1987; Marler, Peters, Ball, Dufty, and Wingfield, 1988). In the past two decades, the mechanisms by which testosterone promotes these changes have become clearer (e.g., Gurney and Konishi, 1980; Nottebohm, 1980; Brenowitz and Arnold, 1992; Schlinger and Arnold, 1992). Testosterone affects central and peripheral targets, including the growth of vocal control nuclei in the brain (DeVoogd, 1987, 1991) and of syringeal musculature related to song production (Luine, Nottebohm, Harding, and McEwen, 1980). These changes can be induced in nonbreeding birds, which have low levels of testosterone, by systemic administration of testosterone (e.g., Nottebohm, 1980; Nowicki and Ball, 1989).

Although these findings show that testosterone is critical to the development and expression of song, previous work on the timing of song development suggests a potential cost of elevating testosterone early in life. Administration of testosterone during early phases of

<sup>1</sup> To whom correspondence should be addressed. Fax: (812) 855-6705. E-mail: rtitus@indiana.edu.

song development, when levels are normally low, caused acoustical abnormalities in the songs of zebra finches (*Taeniopygia guttata*) (Korsia and Bottjer, 1991), in which learning and production phases of song development overlap in part. By beginning testosterone treatment of young males at several different ages (the latest during subsong production), Korsia and Bottjer found that temporal, structural, and syntactical features of song differed in the timing of their susceptibility to altered testosterone levels. In a similar study, Whaling, Nelson, and Marler (1995) delayed testosterone treatment of white-crowned sparrows (*Zonotrichia leucophrys*) until after birds had memorized song (the memorization phase of song development, which in this species occurs early in life), but administered the hormone before the sparrows had begun practicing song (the sensorimotor phase, which normally occurs many months later; Marler, 1984). They found that songs of treated birds were loosely structured, resembling those of birds raised in isolation (Konishi, 1964a). Similar results were reported by Volman (1993) for the one sparrow tested.

To extend this line of questioning, in the present study we administered testosterone to dark-eyed juncos at a still later phase in song development, after the sensorimotor phase was well advanced but before song crystallization was completed (plastic song phase). By administering exogenous testosterone to yearling males after they had begun to sing and then measuring song production and structure, we thus allowed ontogeny to proceed normally beyond the phases addressed by previous studies. The storage phase (between memorization and production) presumably was complete or nearly so, and production (i.e., motor learning) stages of song had begun. In juncos, full song develops after periods of subsong and plastic song in the early spring (Marler, Kreith, and Tamura, 1962). Although the durations of these periods are not known, in related sparrows they are approximately 1 to 2 months in length (Marler *et al.*, 1988).

Because experimentally elevated testosterone is associated with increased song production, we predicted that song production in testosterone-treated groups would exceed that in controls. We also predicted that repertoire size would be greater, either because song was more frequent or possibly because testosterone might influence the development or expression of larger song repertoires (i.e., more song types per male).

## METHODS

### *Animals and Housing Conditions*

Male juncos (all ca. 120–170 days old, aged and sexed by eye color and plumage characteristics; Ket-

terson and Nolan, 1976, 1982; Yunick, 1981; Mulvihill and Chandler, 1991) were captured during October and November 1991 at or near Mountain Lake Biological Station, Giles Co., Virginia, and were transported to Bloomington, Indiana, where they were housed together outdoors in an aviary (7 × 4 × 3 m). On 21 November, 52 males were moved into the Animal Quarters at Indiana University, where they were housed continuously in individual cages (29 × 23 × 26 cm) in observation rooms (1.5 × 1.5 × 2.3 m), with food and water provided *ad libitum*. Observation rooms 1 and 2 contained 15 birds each, while room 3 held the remaining 22 birds. In all rooms, cages were aligned along one wall and arranged so that birds were visually but not acoustically isolated. Temperature was approximately 16°C, and lights were maintained on a 8.5L:15.5D cycle until 28 December. On that day, photoperiod was increased by 1 hr every other day until 7 January, when it reached a 14.5L:9.5D hr cycle, designed to promote the onset of reproductive condition (Follett and Farner, 1966). This cycle was maintained until the experiment ended on 6 May.

### *Implantation with Testosterone*

On 23 January 1992, 16 days after maximum daylength was attained, males were assigned to one of three groups for treatment with implants: controls (C), one dose of testosterone (TI), or two doses of testosterone (TII). Males in rooms 1 and 2 were randomly assigned to one of the 3 treatment groups, while those in room 3 were randomly assigned to C or TII treatments. Thus, rooms 1 and 2 each contained 5 C, 5 TI, and 5 TII males, while room 3 contained 10 C and 12 TII males. Birds were implanted subcutaneously with 10-mm lengths of silastic tubing (Dow Corning, Midland, MI; 1.47 mm ID, 1.96 mm OD), which were empty (C birds) or packed with crystalline testosterone (Sigma Chemical Co., St. Louis, MO; TI birds received one implant and TII birds received two). Tubes were sealed with silastic glue. In the wild, the TII dose induces plasma testosterone levels equivalent to the maximum values exhibited naturally by juncos in early spring (Ketterson and Nolan, 1992; Chandler, Ketterson, and Nolan, in press) and maintains these after natural levels in nontreated males have declined (Ketterson and Nolan, 1992; Chandler *et al.*, 1997). At the time of implanting, all birds had begun to sing. Songs resembled adult songs in structure but had more variable endings and longer intersong intervals (R.C.T., personal observation). This falls within the late plastic song phase of Marler and Peters (1982).

## Hormone Assay

As part of a larger experiment (Cawthorn, Ketterson, and Nolan, unpublished study; Klukowski, Cawthorn, Ketterson, and Nolan, 1997), birds were bled approximately every 3 weeks to ensure that implants had effectively increased testosterone levels and also to monitor corticosterone and corticosterone-binding globulin. In this paper, we report the results of the sampling carried out on 10 March 1992, 4 days after our focal observations were completed. These levels are representative of those throughout the study (Klukowski *et al.*, 1997). This sampling included 10 C, 8 TI, and 10 TII males (7 from rooms 1 and 2 combined, 21 from room 3).

Radioimmunoassay was performed by T. Johnsen to determine plasma concentration of testosterone (procedures followed Wingfield and Farner, 1975; Wingfield, Newmann, Hunt, and Farner, 1982; and Ball and Wingfield, 1987; see Ketterson *et al.*, 1991a). Briefly, blood was spun in a clinical centrifuge, and the plasma fraction was drawn off and stored at  $-20^{\circ}\text{C}$  until assayed. Plasma samples were measured, and a small volume of labeled testosterone was added to enable calculation of recoveries. Steroid hormones were separated from the aqueous phase with dichloromethane prior to separation on celite/glycol columns. Percentage recoveries averaged 79.38% (SE = 0.01) and the intra-assay coefficient of variation was 17%. All samples were run in one assay.

## Focal Observations

Between 26 February and 6 March 1992, we observed males in rooms 1 and 2, selecting those that were most readily visible through observation windows (in all, five C, six TI, and six TII). Each was observed for 5 min on six or seven occasions, for a total of 30–35 min per bird. Observations were made at all times of the day and typically were separated by at least 1 day, but eight birds were observed twice on 1 day and nine other birds were observed three times on another day ( $>1$  hr separated observations of the same bird). Observations began at predetermined times; i.e., we did not wait until birds were singing. Time of observation did not differ among treatment groups ( $F(2, 14) = 0.1870$ ,  $P > 0.05$ ). There were no observations on days on which birds were bled, and treatment of individuals was unknown to the observers until after the study had been completed.

Numbers of songs and of song types during each 5-min observation period were counted for each male, counts for all periods were summed, and the average per minute for each male was used in statistical analy-

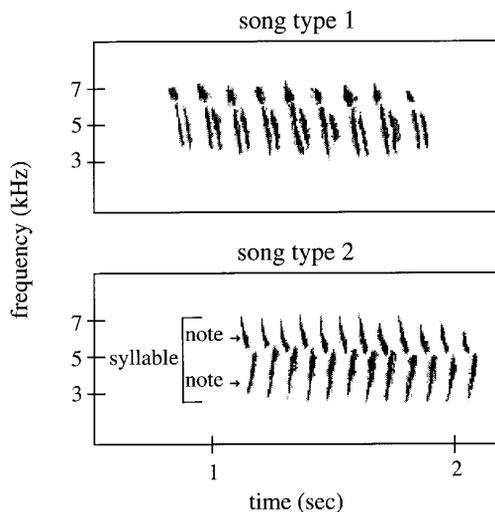


FIG. 1. Sonograms of two song types produced by a male dark-eyed junco. Note the structural variation between the types. Notes and syllables are labeled in the second sonogram.

ses. In this species, songs are discrete units typically composed of one syllable repeated 10–20 times in rapid succession; songs are separated from one another by 2–7 sec of silence (Konishi, 1964b; Williams and MacRoberts, 1977). Each male has a repertoire of several song types, which are easily distinguished aurally as well as by their shapes on sonograms (Fig. 1; Marler *et al.*, 1962; Konishi, 1964b; Williams and MacRoberts, 1977). Written descriptions of each song type were made during each observation and were later compared to provide an estimate of each bird's repertoire size. Songs were accompanied by distinctive backward head movements, making identity of the singer certain (Hostetter, 1961; Konishi, 1964a). During the breeding season, yearling male juncos such as those used in this study produce fully developed song and constitute roughly half of all breeding males in the population from which we obtained our subjects (Ketterson, Nolan, Ziegenfus, Cawthorn, and Cullen, 1991b).

## Recordings

After the focal observations had been completed, to determine whether early treatment with testosterone affected structural characteristics of song, 21 randomly selected males (9 C, 5 TI, 7 TII) were tape-recorded at all times of the day between 6 April and 6 May 1992. Five of these males (1 C, 3 TI, 1 TII) had participated in the focal study. One male at a time was moved from its home cage into a separate room where it was recorded 5–60 min later using a Sennheiser ME30 micro-

phone attached to a Marantz 221 cassette recorder. We made two 45-min recordings of each male. Overall, about half the males tested sang during recording sessions: 3 C males (which sang 39, 284, and 514 songs), 4 TI males (53, 59, 195, and 795 songs) and 4 TII males (23, 124, 147, and 273 songs). The time of day and length of time males spent in the recording room had no effect on the number of songs produced (Pearson correlation coefficients =  $-0.193$  and  $0.144$ , respectively).

Using a Uniscan II sound spectral analyzer, the first five examples of each song type in each male's repertoire were printed. The following structural qualities were measured: frequency range (the difference between the highest and the lowest frequencies within a song); number of syllables per second (syllables are the largest repeated units within songs and are readily distinguished on sonagrams; see Fig. 1; Konishi, 1964b); and number of notes (continuous tracings on sonagrams; see Fig. 1) per syllable. We also assessed stereotypy, which is a subjective measure of the degree of repetition of a song type; it is based on such features as number of syllables per song and the modulation of both frequency and amplitude (Korsia and Bottjer, 1991). For each bird that sang, the degree of stereotypy was determined by two observers who were unfamiliar with the study and who listened to all the tapes in random order. Each observer assigned each tape a score from least (1) to most (5) stereotyped (after Korsia and Bottjer, 1991). On 10 of the 11 tapes observers assigned scores that were within 1 point of each other (for example, scores of 4 and 5 or 5 and 5), while for the remaining tape their scores differed by 2 points. The average of the two observers' values for each tape was used for comparisons.

### Statistical Analysis

Song rates of males during the focal observations were normally distributed within treatment groups (Lilliefors test;  $D = 0.187-0.329$ ,  $P = 0.081-1.00$ ), but number of song types was not (Lilliefors test;  $D = 0.272-0.500$ ,  $P = 0.00-0.33$ ). To quantify the effect of treatment on song rates, we therefore used one-way analysis of variance (ANOVA) followed by a *post hoc* Bonferroni test, and to compare treatment effect on number of song types we used the Kruskal-Wallis test followed by Mann-Whitney *U* tests adjusted for multiple comparisons (Siegel and Castellan, 1988). All probabilities associated with parametric tests are two-tailed, and means are presented  $\pm$  one standard error. Structural data on the relatively small number of males that sang during recording sessions were not compared statistically.

## RESULTS

### Testosterone Levels of Birds in the Three Groups

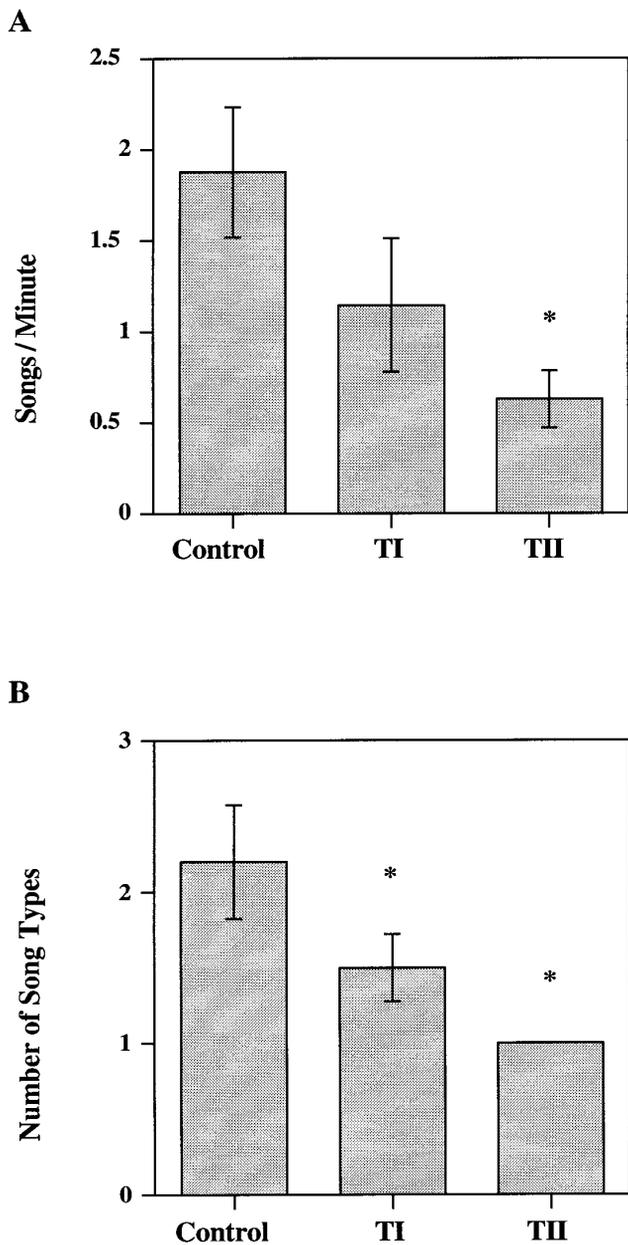
Males in the TII group had the highest testosterone levels ( $8.9 \pm 0.9$  ng/ml,  $n = 10$ ), TI males had intermediate levels ( $4.8 \pm 0.8$ ,  $n = 8$ ), and C males had the lowest levels ( $0.9 \pm 0.3$ ,  $n = 10$ ;  $F(2, 25) = 32.89$ ,  $P < 0.001$ ; *post hoc t* tests showed that all three groups differed from one another at  $P < 0.01$ ). Levels among testosterone-treated males were similar to those achieved by birds in the wild when near their maxima; levels in controls were similar to those seen before and after the early spring peak (Ketterson *et al.*, 1991a; Ketterson and Nolan, 1992). Seven of these 28 RIA-assayed males had been observed as part of the focal study. Previous work has shown that implants of this size tonically release testosterone for months (Ketterson and Nolan, 1992; Nolan, Ketterson, Ziegenfus, Cullen, and Chandler, 1992).

### Focal Observations

Seventeen males were observed 30–35 min each for a grand total of 545 min of observation time. Each male sang during at least one 5-min observation period; overall, males sang during half (54 of 109) of the periods (C males sang during 51% of observations, TI males sang during 53% of observations, and TII males sang during 47% of observations). Song rate (the mean number of songs produced per minute) differed among the three groups (Fig. 2A; mean for C males  $2.5 \pm 0.4$  songs, for TI males  $1.1 \pm 0.4$  songs, and for TII males  $0.6 \pm 0.2$  songs;  $F(2, 14) = 4.066$ ,  $P < 0.05$ ); *post hoc* tests showed that C and TII males differed significantly (Bonferroni test,  $P = 0.039$ ). Similarly, the number of song types an individual produced differed among treatment groups (Fig. 2B; the mean for C males was  $2.2 \pm 0.4$  song types, the mean for TI males was  $1.5 \pm 0.2$  song types, and the mean for TII males was  $1.0 \pm 0.0$  song types,  $P = 0.033$ ; *post hoc* tests indicated that C and TII males, and TI and TII males, differed from one another;  $P < 0.05$ ; C and TI males did not differ significantly).

### Structure of Songs

Because recordings were made considerably later in the year than focal observations and after many of the controls had ceased to sing, data obtained from the recordings are not sufficient to allow statistical comparisons that have much power to detect differences (power of all tests  $< 20\%$  at the  $P = 0.05$  level). Number of notes per syllable (C,  $2.6 \pm 0.3$ ; TI,  $3.1 \pm 0.2$ ; TII, 2.8



**FIG. 2.** (A) Mean song production (songs/min)  $\pm$  SEM by captive yearling male dark-eyed juncos according to treatment. Group C, controls ( $n = 5$ ), received empty 10-mm implants; group TI ( $n = 6$ ) received one 10-mm implant of packed crystalline testosterone; group TII ( $n = 6$ ) received two 10-mm testosterone implants. Song production ca. 40 days after treatment was lower in groups with higher dosages of testosterone. (B) Mean number of song types  $\pm$  SEM produced by yearling male dark-eyed juncos during focal observations; treatment groups defined as in (A). Song showed a significant decline with increasing testosterone level of the groups; \*Significantly different from controls,  $P < 0.05$ .

$\pm 0.2$ ) and frequency range (C males,  $n = 3$ ,  $2956 \pm 251$  Hz; TI males,  $n = 4$ ,  $3379 \pm 245$ ; TII males,  $n = 4$ ,  $2841 \pm 217$ ) showed overlapping distributions, as did

stereotypy scores of  $4.2 \pm 0.6$  for C males,  $4.6 \pm 0.2$  for TI males, and  $4.1 \pm 0.5$  for TII males.

Two song features related to timing showed parallel trends. There was some suggestion that males in testosterone-implanted groups produced more syllables per second than controls (C,  $7.2 \pm 0.4$ ; TI,  $9.2 \pm 0.4$ ; TII,  $8.7 \pm 0.98$ ). Time between songs also appeared diminished for testosterone-implanted birds. Control males averaged  $5.13 \pm 1.44$  sec between successive songs (range 3.25–7.96), TI males  $4.22 \pm 0.94$  (range 1.97–6.37), and TII males  $4.10 \pm 0.67$  (range 1.86–6.27). Differences remained nonsignificant when both testosterone-treated groups were lumped and compared as a group with controls.

## DISCUSSION

Contrary to our expectations, captive yearling male juncos implanted with testosterone produced significantly fewer songs and expressed significantly smaller song repertoires than did controls. Thus, experimentally elevated testosterone levels during the late sensorimotor phase of song learning (i.e., that in which the bird practices and refines its song) caused significant changes in singing behavior. Previous studies have reported that early exposure to high testosterone can alter song structure (Whaling *et al.*, 1995; Korsia and Bottjer, 1991). We add here that frequency of singing, normally positively associated with testosterone, is also subject to perturbation. Taken together, these results are surprising because the majority of field and laboratory studies have found that testosterone increases song production (reviewed in Balthazart, 1983; Pröve, 1983; for juncos see Ketterson *et al.*, 1992; Chandler *et al.*, 1994). Related studies discussed below suggest that a possible cause of these differences may have been disruption of normal patterns of song learning.

Among previous investigators who have examined the role of testosterone in song development, Marler *et al.* (1988) found that the presence or absence of testosterone during certain stages of song learning was critical to normal song development in the swamp sparrow (*Melospiza georgiana*). Castrated male sparrows did not sing normally structured songs, but they could be induced to produce such songs later in life using testosterone therapy. Testosterone also hastens the onset of song crystallization (Arnold, 1975; Kroodsma, 1986; Nottebohm *et al.*, 1987) in zebra finches, marsh wrens (*Cistothorus palustris*), and canaries (*Serinus canaria*).

Two studies closely resemble ours in their approaches to testosterone and the timing of its role in song development. Korsia and Bottjer (1991) investi-

gated the ontogeny of different features of song in zebra finches by beginning testosterone treatment during memorization and early sensorimotor phases (up to approximately 40 days of age in the species studied). They suggested that high testosterone levels at inappropriate (i.e., premature) stages of development inhibit proper song learning and subsequent song production. Whaling *et al.* (1995) began treatment of white-crowned sparrows at later developmental stages and found that administration of testosterone after the memorization phase but prior to the sensorimotor phase of song learning result in abnormally structured song. Males in our study were first subjected to testosterone in the plastic-song phase, which occurred after treatment began for the oldest group of finches tested. Though limited, our structural data contrast in critical ways with the results of these previous studies. We found that treatment of juncos with testosterone later in development but prior to crystallization did not appear to strongly affect song structure. Rather, songs of testosterone-implanted males and control males shared overlapping ranges for all song measurements. Further, inspection of sonagrams showed that structure also overlapped with songs of normal free-ranging males (see Konishi, 1964b; Williams and MacRoberts, 1977 for measurements of songs of free-ranging male juncos).

Korsia and Bottjer (1991) suggest that temporal features of song develop later than do structural features. When zebra finches were administered testosterone prior to the period of plastic song production, the number of song phrases per bout (a temporal feature) increased, although not in all cases. Our limited data also suggest that elevation of testosterone levels late in song development may have similar effects on temporal features of song. Although songs of testosterone-treated male juncos were within normal ranges for the features we measured, they tended to contain more syllables per second and to be closer together in time. More studies that administer exogenous hormones during development and thereby disrupt normal hormonal profiles (following Korsia and Bottjer, 1991) would add to our growing understanding of the physiological mechanisms underlying song learning.

Turning to the role of natural selection in shaping schedules of development, there are several reasons to think that high testosterone levels early in development would be advantageous in some respects. For example, one possible benefit to early elevation of testosterone might be earlier reproduction. Field data and theory from several studies suggest that young produced early in the breeding season have higher reproductive value, suggesting that selection should favor early breeding (Kroodsma and Pickert, 1980; Arcese and Smith, 1985).

Despite possible benefits of high testosterone levels for other aspects of development and fitness, however, the potential influence of testosterone in reducing singing and possibly altering song learning may constrain early elevation of testosterone levels. The constraints acting to limit early breeding are usually thought to be environmental and energetic, e.g., early breeding poses risks of starvation of young or high metabolic costs. Our study suggests that normal development of the song system may pose a constraint on the degree to which males can deviate in time of breeding from an existing developmental schedule.

Consistent with this view is the fact that testosterone levels in other species studied are low during learning stages of development, rising slowly as song crystallization occurs (Pröve, 1983; Marler, Peters, and Wingfield, 1987). For example, adult canaries cease adding new syllables to their repertoires when testosterone levels reach spring maxima (Nottebohm *et al.*, 1987). Similarly, the finding that yearling male juncos have lower testosterone levels than do older males (Ketterson and Nolan, 1992) is consistent with the supposition that high testosterone levels might inhibit the learning that allows young males to crystallize songs at the appropriate time in development. In captivity, male juncos copy songs from other males with which they interact, and as adults they continue to modify and add new song types to their repertoires (Marler *et al.*, 1962; personal observation). Whether free-living juncos also modify song repertoires after the first breeding season is not known. Small-scale modifications of songs to fit local acoustical and social environments may require the flexibility enabled by the naturally slow rise in testosterone levels (Baptista, 1996). The crystallization apparently induced by high testosterone may mark the end of a bird's ability to modify song structure, at least for that breeding season.

## ACKNOWLEDGMENTS

This study was funded by the Department of Biology, Indiana University, the Indiana Academy of Science, a Research Training Grant from the NSF (DIR-9014276), and NSF Grant IBN-9111498 to E.K. and V.N. We thank C. Ray Chandler, Samrrah Raouf, and Lori Klukowski for capturing and transporting birds, and the staff of University of Virginia's Mountain Lake Biological Station and the staff of Mountain Lake Hotel for use of facilities while capturing birds. We also thank Torgier Johnsen for performing the hormone assays. We greatly appreciate the assistance of Michelle Cawthorn for use of the birds and Lori Klukowski and Holly Graf for their care of the birds. Tracey Kast and Daniela Monk assessed stereotypy of songs. C. Alex Buerkle, Tracey Kast, Matt Klukowski, Samrrah Raouf, Steve Schoech, and three anonymous reviewers provided helpful comments on this article.

## REFERENCES

- Arcese, P., and Smith, J. (1985). Phenotypic correlates and ecological consequences of dominance in song sparrows. *J. Anim. Ecol.* **57**, 119–136.
- Arnold, A. P. (1975). The effects of castration and androgen replacement on song, courtship, and aggression in zebra finches, (*Poephila guttata*). *J. Exp. Zool.* **191**, 309–326.
- Ball, G. F., and Wingfield, J. C. (1987). Changes in plasma levels of sex steroids in relation to multiple broodedness and nest site density in male starlings. *Physiol. Zool.* **60**, 191–199.
- Balthazart, J. (1983). Hormonal correlates of behavior. In D. S. Farner, J. R. King, and K. C. Parkes (Eds.), *Avian Biology*, Vol. III, pp. 221–365. Academic Press, New York.
- Baptista, L. F. (1996). The nature and nurture of bird song. In D. Kroodsma and E. Miller (Eds.), *The Ecology and Evolution of Acoustic Communication in Birds*, pp. 21–36. Comstock Press, Ithaca, NY.
- Brenowitz, E. A., and Arnold, A. P. (1992). Hormone accumulation in song regions of the canary brain. *J. Neurobiol.* **23**, 871–880.
- Chandler, C. R., Ketterson, E. D., Nolan, V., Jr., and Ziegenfus, C. (1994). Effects of testosterone on spatial activity in free-ranging male dark-eyed juncos, *Junco hyemalis*. *Anim. Behav.* **47**, 1445–1455.
- Chandler, C. R., Ketterson, E. D., and Nolan, V., Jr. (1997). Effects of testosterone on use of space by male dark-eyed juncos, *Junco hyemalis*, when their mates are fertile. *Anim. Behav.* **54**.
- DeVoogd, T. J. (1987). Steroid interactions with structure and function of avian song control regions. *J. Neurobiol.* **17**, 177–201.
- DeVoogd, T. J. (1991). Endocrine modulation of the development and adult function of the avian song system. *Psychoneuroendocrinology* **16**, 41–66.
- Follett, B. K., and Farner, D. S. (1966). The effects of the daily photoperiod on gonadal growth, neurohypophysial hormone content, and neurosecretion in the hypothalamo-hypophysial system of the Japanese quail (*Coturnix coturnix japonica*). *Gen. Comp. Endocrinol.* **7**, 111–124.
- Gurney, M., and Konishi, M. (1980). Hormone-induced sexual differentiation of brain and behavior in the zebra finch. *Science* **208**, 1380–1383.
- Hegner, R. E., and Wingfield, J. C. (1987). Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. *Auk* **104**, 470–480.
- Hostetter, D. R. (1961). Life history of the Carolina junco *Junco hyemalis carolinensis* Brewster. *Raven* **32**, 97–130.
- Ketterson, E. D., and Nolan, V., Jr. (1976). Geographic variation and its climatic correlates in the sex ratio of eastern-wintering dark-eyed juncos. *Ecology* **57**, 679–693.
- Ketterson, E. D., and Nolan, V., Jr. (1982). The role of migration and winter mortality in the life history of a temperate-zone migrant, the dark-eyed junco, as determined from demographic analyses of winter populations. *Auk* **99**, 243–259.
- Ketterson, E. D., and Nolan, V., Jr. (1992). Hormones and life histories: An integrative approach. *Am. Nat.* **140**, S33–S62.
- Ketterson, E. D., Nolan, V., Jr., Cawthorn, M. J., Parker, P. G., and Ziegenfus, C. (1996). Phenotypic engineering: Using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. *Ibis* **138**, 70–86.
- Ketterson, E. D., Nolan, V., Jr., Wolf, L., and Ziegenfus, C. (1992). Testosterone and avian life histories: The effect of experimentally elevated testosterone on behavior and correlates of fitness in the dark-eyed junco (*Junco hyemalis*). *Am. Nat.* **140**, 980–999.
- Ketterson, E. D., Nolan, V., Jr., Wolf, L., Ziegenfus, C., Dufty, A., Jr., Ball, G. F., and Johnsen, T. S. (1991a). Testosterone and avian life histories: The effect of experimentally elevated testosterone on corticosterone and body mass in dark-eyed juncos. *Horm. Behav.* **25**, 489–503.
- Ketterson, E. D., Nolan, V., Jr., Ziegenfus, C., Cawthorn, M., and Cullen, D. P. (1991b). Nonbreeding season attributes of male dark-eyed juncos that acquired breeding territories in their first year. *Acta XX Congr. Int. Ornithol.* **2**, 1229–1239.
- Klukowski, L., Cawthorn, J. M., Ketterson, E. D., and Nolan, V., Jr. (1997). Effects of testosterone on corticosterone and corticosterone binding globulin in captive dark-eyed juncos (*Junco hyemalis*). *Gen. Comp. Endocrinol.* **107**.
- Konishi, M. (1964a). Effects of deafening on song development in two species of juncos. *Condor* **66**, 85–102.
- Konishi, M. (1964b). Song variation in a population of Oregon juncos. *Condor* **66**, 423–436.
- Korsia, S., and Bottjer, S. W. (1991). Chronic testosterone treatment impairs vocal learning in male zebra finches during a restricted period of development. *J. Neurosci.* **11**, 2362–2371.
- Kroodsma, D. E. (1986). Song development in castrated marsh wrens. *Anim. Behav.* **32**, 1572–1575.
- Kroodsma, D. E., and Pickert, R. (1980). Environmentally dependent sensitive periods for avian vocal learning. *Nature* **228**, 477–479.
- Luine, V., Nottebohm, F., Harding, C., and McEwen, B. (1980). Androgen affects cholinergic enzymes in syringeal motor neurons and muscle. *Brain Res.* **192**, 89–107.
- Marler, P. (1984). Song learning: Innate species differences in the learning process. In P. Marler and H. S. Terrace (Eds.), *The Biology of Learning*, pp. 289–310. Dahlem Konf, Berlin.
- Marler, P., Kreith, M., and Tamura, M. (1962). Song development in hand-raised Oregon juncos. *Auk* **79**, 12–30.
- Marler, P., and Peters, S. (1982). Long-term storage of learned bird-song prior to production. *Anim. Behav.* **30**, 479–482.
- Marler, P., Peters, S., Ball, G., Dufty, A. M., Jr., and Wingfield, J. C. (1988). The role of sex steroids in the acquisition and production of birdsong. *Nature* **336**, 770–772.
- Marler, P., Peters, S., and Wingfield, J. C. (1987). Correlations between song acquisition, song production, and plasma levels of testosterone and estradiol in sparrows. *J. Neurobiol.* **18**, 531–547.
- Mulvihill, R. S., and Chandler, C. R. (1991). A comparison of wing shape between migratory and sedentary dark-eyed juncos (*Junco hyemalis*). *Condor* **93**, 172–175.
- Nolan, V., Jr., Ketterson, E. D., Ziegenfus, C., Cullen, D. P., and Chandler, C. R. (1992). Testosterone and avian life histories: Effects of experimentally elevated testosterone on prebasic molt and survival in male dark-eyed juncos. *Condor* **94**, 364–370.
- Nottebohm, F. (1980). Testosterone triggers growth of brain vocal control nuclei in adult female canaries. *Brain Res.* **189**, 429–436.
- Nottebohm, F., Nottebohm, M. E., Crane, L. A., and Wingfield, J. C. (1987). Seasonal changes in gonadal hormone levels of adult male canaries and their relation to song. *Behav. Neural Biol.* **47**, 197–211.
- Nowicki, S., and Ball, G. F. (1989). Testosterone induction of song in photosensitive and photorefractory male sparrows. *Horm. Behav.* **23**, 514–525.
- Pröve, E. (1983). Hormonal correlates of behavioural development in male zebra finches. In J. Balthazart, E. Pröve, and R. Gilles (Eds.), *Hormones and Behaviour in Higher Vertebrates*, pp. 268–274. Springer-Verlag, Berlin.
- Schlinger, B., and Arnold, A. P. (1992). Plasma sex steroids and tissue aromatization in hatchling zebra finches: Implications for the sexual differentiation of singing behavior. *Endocrinology* **140**, 289–299.

- Siegel, S., and Castellan, N. J., Jr. (1988). *Non-parametric Statistics for the Behavioral Sciences*, 2nd ed. McGraw-Hill, New York.
- Silverin, B. (1980). Effects of long-acting testosterone treatment on free-living pied flycatchers, *Ficedula hypoleuca*, during the breeding period. *Anim. Behav.* **28**, 906–912.
- Volman, S. F. (1993). Development of neural selectivity for birdsong during vocal learning. *J. Neurosci.* **13**, 4737–4747.
- Whaling, C. S., Nelson, D. A., and Marler, P. (1995). Testosterone-induced shortening of the storage phase of song development in birds interferes with vocal learning. *Dev. Psychobiol.* **28**, 367–376.
- Williams, L., and MacRoberts, M. (1977). Individual variation in songs of dark-eyed juncos. *Condor* **79**, 106–112.
- Wingfield, J. C., and Farner, D. S. (1975). The determination of five steroids in avian plasma by radioimmunoassay and competitive protein binding. *Steroids* **26**, 311–327.
- Wingfield, J. C., Newmann, A., Hunt, G. L., and Farner, D. S. (1982). Physiologic properties of steroid hormone-binding proteins in avian blood. *Gen. Comp. Endocrinol.* **53**, 281–292.
- Yunick, R. P. (1981). Age determination of winter and spring dark-eyed juncos. *N. Am. Bird Bander* **6**, 97.