

TESTOSTERONE AND AVIAN LIFE HISTORIES: EFFECTS OF EXPERIMENTALLY ELEVATED TESTOSTERONE ON PREBASIC MOLT AND SURVIVAL IN MALE DARK-EYED JUNCOS¹

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Abstract. Male Dark-eyed Juncos (*Junco hyemalis*) that breed in Virginia begin their prebasic molt after breeding has ended, usually in August. Almost all males caught in late October have completed the molt. In 1989, we obtained anecdotal evidence that males whose testosterone (T) we maintained at artificially elevated levels beyond the end of the breeding season postponed or suppressed prebasic molt. To test the effect of T experimentally, in spring 1990 we implanted some males (T-males) with testosterone and others (C-males) with empty implants, and we released both groups to breed. We caught some members of both treatment groups in October and removed their implants. The T-males had delayed their prebasic molt, while the C-males were molting on schedule. Other implanted T- and C-males were not caught; these carried their implants into winter. Next spring we examined surviving males whose implants we had removed in October as well as males whose T- and C-implants had not been removed. T-males whose implants we had removed had molted completely, despite their delayed start, whereas T-males whose implants we had not removed had not molted. Still-implanted C-males had molted. We compared the minimum overwinter survival (i.e., return rates in spring) of the treatment groups. T- and C-males whose implants we had removed in October returned at the same rate, but among males whose implants we had not removed, significantly fewer T-males than C-males returned.

The transition between reproduction and molt of male juncos apparently can be blocked by preventing the normal seasonal decline in T. This suggests a physiological basis for a possible trade-off between time allocated to reproduction and time allocated to molt. Our results indicate that males could maintain high T and prolong breeding, possibly into October, and still molt completely with no adverse effects. We consider why such a modification of schedule has not occurred. However, postponement of molt beyond some date in autumn, possibly late October, suppresses it altogether, as indicated by the failure to molt of the returning T-males whose implants we did not remove. This treatment group apparently suffered higher overwinter mortality, and we consider possible reasons.

Key words: Junco; testosterone; prebasic molt; evolutionary trade-offs; molt; termination of reproduction; termination of molt.

INTRODUCTION

In many seasonally breeding bird species, energy-consuming functions such as reproduction, molt, and migration tend to occur sequentially (Amadon 1966, review in Payne 1972, Farner 1983), with little or no overlap. In such species, time and energy expended on one function may be at the expense of time and energy available for others. If a single physiological mechanism

stimulates one function while suppressing another, selection could operate on the timing of the mechanism, resulting in an evolutionary trade-off that balances the costs and benefits of shifts in this timing.

Testosterone (T) is critical to avian reproduction, and it has long been known that its decline at the end of the breeding season is in some species implicated in the transition into molt (Payne 1972; for recent studies, see Schlessner et al. 1985, Runfeldt and Wingfield 1985, Morton et al. 1990). By treating males with testosterone it is possible to investigate experimentally the po-

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tential fitness consequences of prolonging reproduction and delaying molt. In the one field study that we are aware of, administration of testosterone to Song Sparrows (*Melospiza melodia*) prolonged reproductive condition and postponed molt (Runfeldt and Wingfield 1985), but the fitness consequences of delaying molt were not investigated.

In 1989, in an experiment conducted on breeding male Dark-eyed Juncos (*Junco hyemalis*), we obtained anecdotal evidence that males implanted with exogenous T delayed prebasic molt. Therefore in 1990 we tested the hypothesis that elevated testosterone delays and even suppresses molt in male juncos, depending on the date on which the testosterone implant is removed. Furthermore, we predicted that the consequences of abnormal molt would reduce the survivorship of T-males below that of males with empty implants (controls, C-males). If the prediction proved true, which it did, this would indicate that male juncos would gain no selective advantage by trading off time currently devoted to molt in favor of a prolonged breeding season. The basis for our prediction was the view that the existing molt schedule might constrain the breeding schedule and be responsible for the termination of breeding (compare Pitelka 1958, Miller 1961), but our results indicate that this view was mistaken. More likely, juncos quit breeding at a time when the reproductive value of any young they might produce would not counterbalance the necessary parental effort.

METHODS

STUDY POPULATION

Our study site is the Mountain Lake Biological Station in Giles County, Virginia (described in Nolan et al. 1986, Wolf 1987). In juncos that breed at high elevations in the southern Allegheny Mountains ("Carolina" juncos), some individuals move short distances downslope in autumn or early winter, but some, usually adult males (i.e., more than one year old), remain on the breeding ground (Nolan et al. 1986, Ketterson et al. 1991b). Males become territorial in March and April, and survivors (50%–60% each year) virtually always return to the territory of the preceding year (unpubl. data). There is little or no prealternate molt. The mating system is apparently monogamous; females build nests and incubate, and males share in the feeding of nest-

lings and fledglings (Wolf et al. 1990). Egg-laying lasts from late April until, in some cases, early August. Pairs occasionally raise as many as three broods, but often they spend the entire season attempting to raise a single brood in the face of heavy nest predation. All individuals referred to herein were uniquely color-banded.

PREBASIC MOLT

Molt of the alar tract spans almost the full time required for all the remaining tracts to molt, so that quantifying the state of an individual's primaries provides a good estimate of the overall progress of its molt. As is typical of many passerines, juncos drop the innermost primary first, and thereafter at intervals of several days drop primaries 2 through 9, in that order. Shortly after an old feather is lost, its replacement appears and gradually develops. Thus, a junco in mid-molt of the primaries might have feathers 1–3 fully renewed, replacements 4–6 still growing (in length, $4 > 5 > 6$), 7 missing, and old feathers 8 and 9 still present. We quantified primary molt using Nolan's modification (1978, Table 184) of Newton's (1966) method. We scored each fully grown new primary on the right wing as 10 and each old primary as 0; a gap was scored as 0.5, and a growing new feather was scored according to its length in proportion to the average length of a full-grown replacement (e.g., 10% of full length = 1, 90% = 9). Extreme scores therefore were 0 and 90.

Molt normally begins in August or September and usually ends by late October. Thus, among 37 unmanipulated adult males examined in the hand in autumn (15–22 October 1986, 12–19 October 1987, 2–14 October 1989, 6–16 October 1990, and 27 October–2 November 1991), 32 had completed primary molt. Molt of the others was nearly complete, except for one male whose primary score was 76.

EXPERIMENTAL METHODS

In 1989 (7 March–30 April) and 1990 (9 April–1 June, but mostly in April) we caught, color-banded (unless they were already banded), anesthetized (methoxyfluorane), and implanted males with one 15-mm and one 5-mm length of silastic tubing, sealed at each end with silastic glue (Dow Corning; i.d. 0.58 mm, o.d. 0.77 mm). In T-males, the tubing was packed with crystalline testosterone (Sigma Chemical) and inserted subcutaneously to lie along the left flank. C-males

were treated identically except that their tubing was empty. To determine the effect of the implants on plasma T, we recaptured many subjects in May, June, and early July. Blood samples (100–150 μ l) were drawn and later subjected to radioimmunoassay (RIA) using the methods of Wingfield and Farner (1975) and Wingfield et al. (1982). Testosterone in T-males was at the physiological levels found among free-living unimplanted males during the peak of T that occurs while territories are established in spring (6–9 ng/ml; Ketterson and Nolan, in press). Levels of testosterone in C-males were significantly lower. RIAs in 1989 and earlier years showed that testosterone of T-males remained at the experimentally produced peak for at least 40 days after implanting (Ketterson et al. 1991a).

In 1989 removal of implants from both T- and C-males took place between 17 July and 10 August and (in the case of males not recaptured in July–August) between 2 and 14 October. At least two T-males were not caught at either time and still retained implants the following spring, when the implants were empty. In 1990 we did not remove T- or C-implants until 5–17 October. Males were on or near their territories at that time, and we made our capture efforts on territories selected for accessibility and did not differ from other territories in known respects. We took out the implants of males that we caught and (with one exception) observed their primary scores. Those birds not caught carried their implants into autumn and winter. We encountered many males of both treatment groups in spring 1991. All returning T-males, both those that still carried implants and those whose implants we had removed in October, were recaptured, their plumage examined, and implants (if present) removed.

RETURN RATES

Birds of both treatment groups that were caught in October 1990, whose implants we had removed, could be appropriately compared in a pair-wise test. Because we predicted no difference in the proportions of returning males, we used Fisher's exact test, two-tailed. T- and C-males that we did not catch in October 1990 did not have their implants removed, but we knew they had been alive at least as late as July 1990 (when we caught or saw them). Here we predicted lower survival of T-males and tested our data using a

one-tailed Fisher's exact. Implants sometimes work their way out through the skin, and this comparison of returns of birds whose implants we did not remove is therefore conservative.

RESULTS

MALES IMPLANTED IN 1989

We report these anecdotal results as background for the 1990 experiment. Among 39 adult male juncos captured in October (Table 1) were birds that we had not implanted, T- and C-males whose implants we had removed in summer, nine still-implanted C-males, and two still-implanted T-males that we had failed to catch for implant removal in July–August. Molt of the two T-males with implants was delayed, but molt of the T-males whose implants we had removed in July–August was on the normal schedule (Kruskal-Wallis test comparing primary scores of the five groups in Table 1: $H = 16.056$, two-tailed $P = 0.003$). In Dunn's a posteriori multiple comparisons, the still-implanted T-males differed from the other groups ($P < 0.05$), which did not differ among themselves.

Of the two T-males that we caught in spring 1990 and that still carried implants (see Methods), one had not molted, and abrasion had greatly reduced the barbs of the primaries and shortened the wing (flattened) from 83 mm in 1989 to 76 mm in 1990. The body plumage was worn and exhibited unfeathered gaps, especially on the ventral tract. The second male had renewed some primaries, secondaries, and wing coverts, but the alular coverts had not molted.

For T- and C-males whose implants we had removed in July–August 1989, we compared the return rate in 1990: 12 of 18 T-males and 7 of 15 C-males returned (χ^2 , ns).

MALES IMPLANTED IN 1990

Among males implanted in spring and captured in October 1990 were ten T-males and ten C-males (Fig. 1). Four of the T-males had not begun to molt. Molt of the other T-males, though delayed, was proceeding in normal sequence, i.e., primaries lost centrifugally and other tracts and regions at stages consistent with the state of primary molt. In a Mann-Whitney U -test of primary scores, $U = 90.0$, two-tailed $P < 0.001$. We inspected the implants that we removed from T-males and found they still contained small quantities of testosterone, one only a trace.

TABLE 1. Primary molt score¹ of adult² males, October 1989, according to treatment.

	Not implanted	Empty implant		T-implant	
		Present	Removed	Present	Removed
<i>n</i>	21	9	3	2	4
Median score	90	89	77	35	88
Extremes	76-90	75-90	69-83	25-45	60-90

¹ See Methods.² Adults were juncos that had hatched earlier than 1989 and that were known, or presumed, to have bred in 1989.

In spring 1991, we compared the returns (present in April-May) of C- and T-males according to whether we had removed their implants in October (Fig. 2). In a Fisher's exact test of individuals whose implants we had removed, two-tailed $P = 1.0$. In a Fisher's exact test of individuals whose implants we had not removed, one-tailed $P = 0.036$. Thus the relationship between treatment and return appeared to depend on whether we had removed the implant in October.

All of the five returning T-males whose implants we had removed had molted completely. Two of these were among the four that had not yet begun to molt when we took out their implants (on 5 and 10 October, respectively). The returning T-male that retained his implant in spring had not molted. The implant, which we removed, was empty. (Interestingly, despite his worn and faded plumage, he obtained a mate and reproduced in 1991. He had not previously been mated to this female.) All returning C-males had molted.

DISCUSSION

EFFECT OF T ON MOLT

Twelve T-males (2 in 1989, 10 in 1990) that retained implants until October, when we removed them, delayed their molt but nevertheless molted completely, as we learned in spring. Interestingly, two of these had not begun molt in October but nevertheless completed the process. This finding is inconsistent with the results of Schlessner et al. (1985) for T-implanted male European Starlings (*Sturnus vulgaris*) held in captivity. Starling T-males began to molt at an abnormally late date, after their implants were removed, but they quit molting at about the usual time for the natural, free-living population. As a result of this truncated molt period, the experimental starlings did not molt completely. The authors proposed that termination of molt in starlings is controlled by the timing of the end of photorefractoriness or possibly by an endogenous circannual periodicity.

Although the molt in male juncos, once begun,

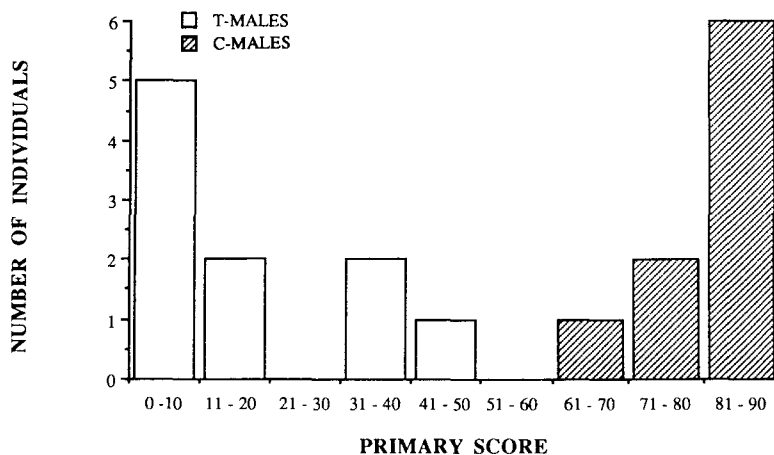


FIGURE 1. Primary molt scores (see Methods) of individual males in October 1990, according to treatment.

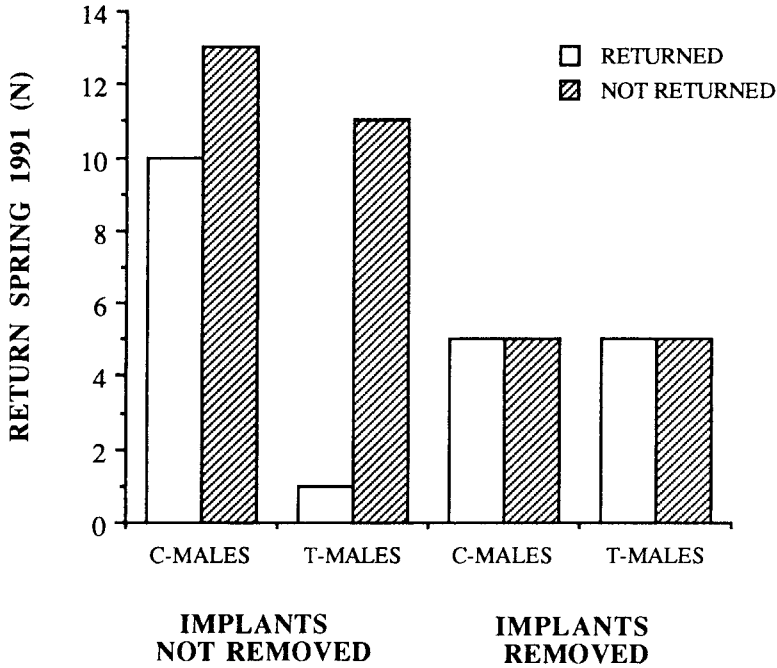


FIGURE 2. Numbers of C- and T-males that were implanted in spring 1990, that were alive in July 1990 (implants not removed) or in October 1990 (implants removed), according to whether they returned in April–May 1991. Note that survival between July and April–May is not directly comparable with survival between October and April–May.

appears to run its complete course even when its onset is delayed in certain cases until October, postponement beyond some date in autumn evidently results in its suppression altogether. We conclude this from the failure to molt of two still-implanted T-males that returned in the spring (1 each in 1989 and 1990), conjecturing that the testosterone in the implants of these birds was exhausted too late to permit molt to begin.

It is interesting that male juncos whose T-implants we removed in July–August 1989 molted on the normal schedule of the natural population (Table 1), even though their testosterone had been high throughout most or all of the breeding season. This suggests that the exogenous T was quickly cleared metabolically after we took out the implants in July and August (Schleussner et al. 1985). It may also suggest that the physiological changes that prepare the follicles of the primary feathers for molt are very rapid, once T falls to natural levels. Alternatively, if the physiological changes are not rapid and take place gradually even in the presence of high T, molt begins quickly after the inhibition of T disap-

pears (as in Moore et al. 1982 “hypothesis 3,” Moore et al. 1985). Either possibility is consistent with field observations of several species: there is considerable individual variation in the time at which reproduction ends and also a close correlation between the timing of the end of reproduction and the onset of molt (Payne 1972: 110, Nolan 1978:516–518, citations and discussion in Runfeldt and Wingfield 1985). We know of no field investigation of correlations between the end of reproduction and the beginning of molt in free-living juncos. However, when we held adult males from a fully migratory Canadian population in captivity on long days (simulating the photoperiod of their breeding site) but did not permit them to breed, they molted about one month earlier than adult males that we caught from the same local population late in, or just after, reproduction (Nolan and Ketterson 1990). This suggests that without the stimulation associated with reproduction juncos molt as soon as they become photorefractory (compare Runfeldt and Wingfield 1985).

LIFE-HISTORY CONSEQUENCES OF ALTERED MOLT

T-males whose molt was merely delayed (i.e., males whose implants we removed in October) returned at the same rates as C-males. The power of this test to detect a difference between small treatment groups is, of course, quite low, but the 50% rate is consistent with our long-term return data (since 1983) derived from untreated adult males (Ketterson et al., in press).

T-males whose implants we did not remove were significantly less likely than still-implanted C-males to return in the year following treatment, which is comparable to Dufty's (1989) report for male Brown-headed Cowbirds (*Molothrus ater*). Dufty did not remove his implants or measure prebasic molt, but the quantity of hormone in the implants and the plasma concentrations of T declined throughout the breeding season, and he believed that T had been exhausted by the normal date for onset of molt. Consequently, he concluded that the implants did not affect molt, and he attributed the low return rate of T-males to the suggestion that during the breeding season T-implants cause greater aggressiveness and risk of injury in cowbirds. In contrast, our implants did affect molt, and it is likely that this accounted for the lower 1991 return rate of T-males whose implants we did not remove in October 1990. We also point out that T-males whose implants we removed in October tended to return at a higher rate (5 of 10) than T-males whose implants we did not remove (1 of 12), a result that cannot be explained by an effect of T on aggressiveness during the breeding season.

However, the effect of T on molt is not the only possible explanation for our findings. We recognize, but discount the possibility, that the association between T and failure to return was caused by higher mortality of T-males between July, when we last saw them (in some cases August and September), and October. The weather is warm and both insects and seeds are abundant at this time, and the condition of T-males whose implants we removed in October appeared to be normal. We do not know whether still-implanted T-males left their territories in winter, joined winter flocks in the usual way, or accumulated normal winter fat reserves (compare Ketterson et al. 1991a). Failure to do any or all of these

might have reduced overwinter survivorship, independently of the effect of T on molt. An obvious experiment to separate out the effects of T on molt from other possible effects would proceed as follows: Let prebasic molt take place on schedule, capture completely molted juncos at the end of October, insert implants, and determine return rates the following spring. Direct observations of behavior of these birds during winter, if possible, would add information.

The fact that T-males molted completely after implant removal in October 1990 and returned in spring at the normal rate for untreated males brings us back to the subject of possible trade-offs between time for reproduction and for molt. If our results are representative and can be extrapolated to hypothetical males whose endogenous T remained elevated throughout September, why has selection not acted to produce a longer breeding season? One answer could be that females may not be equally free to alter their molt schedule and that selection on the timing of the female's annual cycle controls the male's timing. Female Carolina juncos winter farther downslope than males (Rabenold and Rabenold 1985, Ketterson et al. 1991b), which could accelerate the optimum time of molt for females (as discussed for latitudinally migratory juncos by Chandler and Mulvihill 1990). We have found in the migratory "northern" population of juncos that females tend to migrate earlier and travel farther southward than males (Ketterson and Nolan 1976, 1983; Nolan and Ketterson 1990), suggesting that they may begin to molt earlier. Another possible answer, which does not propose different selective pressures on males and on females, could be that extension of the breeding season would not increase reproductive success. Young from very late broods would have less time to become familiar with the breeding environment before seeking lower elevations. Their postjuvinal molt would probably begin later (but see Gwinner 1986), which might delay movement to winter quarters, possibly until weather conditions were unfavorable. Such potential disadvantages could lower their reproductive value; late-season fledglings in a variety of species have been thought to be of lower value than fledglings produced early in the year (e.g., Nolan 1978:398-399 and citations; see Krentz et al. 1989). Whatever the ultimate cause(s) of the male junco's timing of the end of repro-

duction, a constraint imposed by the need to begin molting is apparently not a factor.

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