

Ketterson / Nolan Research Group Collection

This document is part of a collection that serves two purposes. First it is a public archive for data and documents resulting from evolutionary, ecological, and behavioral research conducted by the Ketterson-Nolan research group. The focus of the research is an abundant North American songbird, the dark-eyed junco, *Junco hyemalis*, and the primary sources of support have been the National Science Foundation and Indiana University. The research was conducted in collaboration with numerous colleagues and students, and the objective of this site is to preserve not only the published products of the research, but also to document the organization and people that led to the published findings. Second it is a repository for the works of Val Nolan Jr., who studied songbirds in addition to the junco: in particular the prairie warbler, *Dendroica discolor*. This site was originally compiled and organized by Eric Snajdr, Nicole Gerlach, and Ellen Ketterson.

Context Statement

This document was generated as part of a long-term biological research project on a songbird, the dark-eyed junco, conducted by the Ketterson/Nolan research group at Indiana University. For more information, please see IUScholarWorks (<https://scholarworks.iu.edu/dspace/handle/2022/7911>).

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Goals 97: working version, April 30, 1997 - this still needs shaping and pruning

Each year we prepare and monitor the study area by creating T- and C-males, and then we follow their relative reproductive success and survival. This work, done by the group as a whole, allows us to monitor annual variation in the impact of testosterone on components of fitness (EPF rates, predation rates, survival, mass of nestlings at fledging, etc.). To this end, we all implant birds, map territories, find nests, bleed band and weigh nestlings, enter data into the computer, and take a hand at the daily list.

Each year we also pursue sub-projects that often lead to publishable papers or at least lay the groundwork for future studies. These tend to focus on various effects of testosterone on the male phenotype so as to gain greater insight into the multiple effects of T on behavior, physiology, and behavior.

We are prompted in particular this year by measures of two fitness components that demand explanation. First, females mated to T-males have lower reproductive success when measured as the number of fledglings produced per season than do females mated to C-males. Why then are they attracted to T-males (Enstrom in press, in prep., Hill in prep), and why do they pair with them in successive years (Ketterson et al. 1996). Second, young that fledge from the nests of T-males return the next year to the study area at a higher rate than young that fledge from the nests of C-males. How does this come about?

General Methods for comparisons of T- and C-males in 1997

Implanting. We are implanting from the War Spur southward, creating the mix of T- and C-implants. [At first I was going to maintain a T-T study area on WVN north, but decided that with no known person to do the DNA work, it was better to have more treated individuals that could be used for experiments.]

Treatment is assigned at random to the first young adult (yearling) captured at each location (trap or net site) and is alternated thereafter for each bird caught subsequently at the same site for all sites. Among old adults (second year and beyond), unbanded males are not implanted unless we capture them after at least one week has passed since initial capture or we capture them after 1 May. This is because they settle off the study area, and we cannot catch them in late summer to remove their implants. Adults that were treated last year and return are given the same treatment this year as they received last (see document entitled 'implant scheme.')

North of the War Spur parking lot is ceded to Tom Smulders. 714 will be used by Brandi and Steve. 714 beyond the junction with the Road to Butt Mountain can be used to capture lures, birds for KJ, etc. We will treat males until some time between the 7th and 14th of May.

Mapping and nest-finding. Quite soon we will begin to map the study area to determine the locations of all males and their nests and identities of their mates. Later we will measure fledging success, nest loss to predators, and patterns of paternity.

Specific goals for 1997

I. This year we will study some *indirect* effects of T by measuring the impact of treating males on the individuals they associate with, i.e., their mates, their offspring, and possibly their neighbors. We shall determine (A) whether females mated to T-males differ in how they deposit steroids in the yolks of the eggs they lay (Lipar and team), and (B) how the decrease in male parental care caused by treatment with T affects the behavior of females, nestlings, and possibly fledglings (Kennedy, Casto (?), team). (C) If time permits, we shall compare T-levels in unimplanted males from 714 with those of C-males on the study area (Schoech, Van Roo, team).

II. We shall also continue to quantify *direct* phenotypic effects of testosterone on males. Specific objectives include comparisons of T-males and C-males with respect to (A) paternal behavior (feeding of nestlings) (team), (B) response to chipmunk model placed near nest (to complete work by Michelle Cawthorn, team), and (C) hippocampal volume and other features of hippocampal neuroanatomy and cell biology (Smulders).

III. We shall also make several measurements of unmanipulated juncos living off the study area (714). In particular, we shall assess cross-correlation of natural levels of T and behavior at different stages of reproduction in unimplanted males (Van Roo).

IV. Also with unmanipulated juncos, Steve Schoech will determine whether injection of an antibody to the hormone prolactin inhibits feeding behavior in male juncos when compared to rates of feeding by males that receive control serum.

V. In addition we will begin studies of Solitary Vireos that are similar to studies already done on juncos by obtaining plasma samples during incubation to allow comparison of T and Prl in males and females.. We shall also attempt a few T-implants to see whether they disrupt incubation by males as we would expect them too (VanRoo).

VI. In a question prompted by an interest in geographic variation in the appearance of junco subspecies, and thus potentially the interaction of sexual selection and reproductive isolation, we shall ask whether female juncos of the northern sub-species prefer members of their own subspecies over males of the Carolina sub-species and vice-versa (Jones).

VII. Finally we will collect information on demography and continue to compile and analyze already collected data to compare the return rates of males, females, and nestlings, as well as the fidelity of females to their mates.

VIII. Finally, finally, there are several miscellaneous projects that might spark someone's interest and that could be done 'on the side.'

I. Indirect effects of treating males with T - impact on their associates

A. does treatment of male juncos with testosterone influence the concentration of steroids in eggs laid by their mates and/or the sex ratio of the young they produce?

Background, demographic. A higher proportion of young raised by females mated to T-males are captured or seen on the study area in the years after they hatch. This suggests that they show either greater site fidelity or are more likely to survive; but in either case, they are more likely to be recruited to the breeding population.

Background, steroids, hatching asynchrony, and differential allocation. In most avian species studied, because incubation begins prior to the laying of the last egg, the last egg to be laid is the last to hatch. The fact that last laid eggs hatch later often puts the chicks that hatch from those eggs at a competitive disadvantage with respect to their nestmates.

In species in which broods are only occasionally reduced in size by starvation despite delayed hatching of the last egg, last-laid eggs (of the few species that have been studied) tend to have higher concentrations of testosterone than earlier-laid eggs (Schwabl). This enhancement of T has been interpreted as an adaptation to increase the probability that young hatching late will survive. In species in which young that hatch from last-laid eggs *typically* die before nest-leaving (e.g., herons and egrets), yolk levels of testosterone decrease with laying order (Schwabl and Mock)

No studies to date have reported variation in steroid levels in eggs related to the 'quality of a female's mate.'. However, there is a literature on differential allocation (Burley) that suggests that females that are mated to attractive males allocate greater effort to caring for young than females mate to unattractive males (Burley on Zebra finches). The interpretation has been that if attractive males give rise to attractive sons, there is a fitness payoff to females that invest heavily in such sons. In our system, we already know that in terms of feedings per hour, females mated to T-males more than compensate; their rate of feeding tends to be higher than that of control females (Ketterson et al. 1992).

What might these observations suggest about how females mated to T-males might differ in their patterns of steroid deposition in eggs? If females can assess whether a male is likely to be a good or poor provider for nestlings, she might be expected to elevate T in the last-laid egg if her mate has attributes that suggest good provider, and not if his attributes suggest the opposite. That is, females mated to T-males might deposit less T in their eggs, particularly the last laid egg than females mated to C-males.

On the other hand, if females respond physiologically to indicators of male quality that co-vary with testosterone, and if early exposure to T improves offspring quality, then females mated to T-males might deposit more T in their eggs than females mated to C-males.

Background, sex ratio. Based on this same reasoning as that used to explain differential allocation, i.e., that attractive males might be expected to produce attractive sons and that females benefit from producing young belonging to the sex with higher variance in reproductive success, if the young they produce are of high quality, we might predict that females mated to T-males would be more likely to produce sons than daughters (Trivers and Willard, Seychelles's Warblers).

Field methods. For nests found during laying, we will visit the nest each day and mark the eggs according to their order of laying. On the day the last egg is laid, we will collect the clutch and freeze the eggs.

For nests found after the clutch is complete and the laying order cannot be known, we will follow the nest until after the young hatch. As soon as the young are old enough, probably on day 3, we will collect 30 ul of blood for DNA which can be used to determine the sex of the young. We will follow nests until the young leave them and from this information determine the relative production of sons and daughters by mates of T- and C-males.

Laboratory methods. When we return to Bloomington, Joe Lipar will analyze the eggs to determine steroid concentrations. Joe is experienced with the methods, which he adapted from Schwabl. Sex of young will be determined using molecular probes that bind to sex-specific bands in passerine birds (e.g., Bradbury and Griffiths 1997).

B. Does treatment of male juncos with testosterone affect behavior of offspring and mates during the period of dependence? Are effects on female behavior caused by cues from the male or from the offspring?

Objectives. Another class of individuals likely to be affected indirectly by treatment of males with T is their offspring. Also just as treatment of the male may affect female 'decisions' with respect to egg formation, we know that it affect their behavior towards young. But we do not know whether the cues that alter female behavior emanate from the young or from the male.

(1) Effect of treatment of male on behavior of offspring: How do young differ in the vocalizations they deliver upon being fed, and in the amount of food they consume. Are young of T-males hungrier than the young of C-males?

Preliminary study. Last year we began a study of how young of T- and C-males communicate their nutritional needs to adults. From nests of T- and C-males with at least three young six days of age, Erin Kennedy and Shan Bentz removed two young and observed their behavior while the young were held inside an automobile in 'fake nests.' Each such experiment lasted 1 hour and 15 minutes

One nestling was fed at regular intervals, the other deprived of food. Every 20 min, the 'fed nestling' was tapped on the bill and offered food three times. We noted whether it ate and how much food it consumed. We also recorded its vocalizations (true?). The 'deprived nestling' was tapped on the bill at 15 min intervals, and we noted whether it vocalized and recorded the sounds it made. We then returned the nestlings to the nest and recorded the vocalizations that the young made when they first fed by a parent of either sex.

Fed nestlings from T-nests consumed food earlier in an experiment and ate more food over the course of the experiment than fed nestlings from control nests. Deprived nestlings from T-nests vocalized earlier in the experiment and also called more often and more loudly than deprived nestlings from C-nests.

Analysis of the recordings of fed and deprived young revealed no differences in the structure of the calls made by young from T- and C-nests, and only small differences in call structure between young that were fed and deprived. The maximum frequencies of calls made by deprived nestlings at the end of the period of deprivation were higher than the maximum frequency in fed nestlings, and the length of individual begging calls was also somewhat greater (Erin, true??)

From this we concluded that young from T-nests begged more and ate more and hence were likely to be chronically hungrier. Further, we concluded that information regarding nutritional needs of nestlings is not likely to be encoded in frequency attributes of call structure. Rather, such information seems more likely to be relayed by means of features of the calls that we did not measure such as duration of a begging bout and/or by amplitude of the calls.

Objectives for 1997 or beyond. Site under construction, various possibilities, suggestions welcome...

We might repeat the experiment of 1996 and measure amplitude and duration of begging bouts. Or we might repeat it but on younger nestlings to see when 'hunger' develops.'

We might play back the calls of fed and deprived nestlings to measure their effect on parental behavior such as latency to the next feeding. We would expect calls of hungrier young to prompt more frequent feedings. We could play the calls at the same volume and amplitude or we could vary amplitude or duration, or both.

Although one explanation for differences between deprived T- and C-nestlings is that T-nestlings are 'hungrier,' because they feed sooner and eat more than C-nestlings (certainly an acceptable operational definition of hunger), the two classes of young did not differ in body mass when taken from the nest. So we have some concern about whether 'hungrier' is the right term to apply T-nestlings; if they weigh the same, why would they be hungrier?

Joe Casto suggested that T-nestlings might be fed on a more variable schedule than C-nestlings and one thing we hope to learn this year is whether this is true (see below). If food delivery is less predictable for T-nestlings than for C-nestlings, this might cause them to 'work harder' for a reward, i.e., beg more vigorously when being fed than a nestling that was fed more regularly. This suggests learning on the part of the nestlings and could be tested by seeing whether a similar experiment done with younger nestlings would give similar results.

Greater begging for a given level of nutritional need also suggests how females mated to T-males come to compensate for the decreased care their mates provide. If young in T-nest beg more for a given level of need, their female parents may be induced to make up the difference. Said another way, glucose levels or fat reserves might be the same in T- and C- nestlings, yet T-nestlings might beg more intensely because they are fed less regularly. One way to test this might be to switch T- and C-young between nests to see whether C-females confronted with young from T-nests began immediately to feed more frequently than before and vice-versa.

We assume the cues that enhance feeding by females emanate from the young, but it is possible that the female is responding to cues from the male such as song. This might predict that females mated to controls would feed more often if we played back male song on their territories.

The questions in this section interest me a great deal, but the connection between them and the overall aims of the project are not as clear as I would like them to be. Any thoughts?

D. Indirect effects on neighbors: Does having a T-male as a neighbor cause a C-male to have higher T than he otherwise would have?

Objective. To compare levels of T in control males that live among T-males with control males that live off the study area. Wingfield showed for song sparrows that a neighbor's T could elevate the hormone in an unimplanted male, but it is not known whether this effect is general nor whether it applies specifically to juncos

II. Phenotypic effects of T, comparisons of parental behavior of T- and C-males

A. Testosterone and parental behavior - descriptive

Background and objectives. We have known for some time that the rate of feeding young is lower in T-males than controls. But there is much that we do not know about the effect of T on male parental behavior. For example, are there also treatment-related differences in (1) how males structure their time when allocating effort to parental behavior, or (2) diurnal patterns of parental behavior. (3) We may also attempt once again to see whether T- and C-males differ in the amounts or kinds of food they bring to the nest.

1 & 2. Time structuring and diurnal patterns

Objective. One goal is to compare how testosterone affects organization of time during the 'parental phase' by comparing behavior of T- and C-males at the nest. In particular, do T-males compartmentalize their parental behavior differently so that they feed in bouts of frequent feedings followed by long gaps of absence, while C-males feed more regularly. Or asking the same question the perspective of a nestling, is their greater variance in interfeeding intervals when the male parent is a T-male or a C-male? Again with respect to T-males, do they feed more in the afternoon than in the morning and how do their patterns compare to C-males?

Background data. To date we have observations made at nests in 1994, 1995 and 1996 when nestlings were aged day 6 and day 9: We have a minimum of x (Erin?) good watches (normal behavior) at each age and in each treatment. These will be used to compare interfeeding intervals (IFIs) of the two treatment groups, and I hope that this analysis might be done this summer.

The data were collected by observing behavior for 1.5-2.0 hrs in the AM; watches were usually completed by 10:30 or 11:00. Days 6 and 9 are informative because feeding is fairly frequent. Further they represent an interesting comparison because the voices of the young change between these ages from high-pitched wines to raspy calls.

Our watches have always been confined to the morning hours. This leaves open the possibility that T-males actually feed more often than C-males in the afternoon, with the possible result that males of the two treatments feed young at similar rates. This seems unlikely, but needs to be addressed.

3. Food brought by adults - load size

Objective: to determine whether T affects the nature or amount of food items brought by adults. I have suspected that although T-males come less often, they bring larger items. However, it is only a hunch, and it is possible (not likely given the compensation exhibited by females) that the reason we fail to detect a big effect of T on growth of offspring is that T-males bring home loads that are large enough to compensate for their less frequent visits.

Methods: HARD!!!! Observe through telescope have some objects of known size visible from the scope in the background of the nest so can describe in objective units, film at the nest, collar the young, etc..... Steve Schoech gave this a shot last year by observing through the scope and ranking load size from 1-3, but I believe he found no effect of treatment. He should be consulted to determine whether this was due to lack of statistical power or the absence of even a trend.

B. Predator presentations

More later on this, but in brief, present model of chipmunk near nests of T-and C-males when young are 6 days old and quantify time required for males to detect predator.

C. Hippocampal anatomy and function (ask Tom Smulders)

D. Why don't T-males feed their young as frequently as C-males? Are they less 'responsive' to begging calls than C-males in the absence of competing stimuli or are they less responsive in some absolute way?

Objective. The goal is to compare responsiveness of T- and C-males to begging vocalizations.

Method: Present captive males (caught from the field and in the parental phase) with recorded vocalizations of young. My guess is that at least for males that actually have young, this will induce hyperactivity - measure level of response (perch hops/5 min?), and possibly measure whether hearing calls and not being able to respond to them induces some correlate of stress, i.e., cort or readiness to pick up food.

So what I picture is capturing male at nest in the PM, holding him overnight, playing calls to him in the AM with an alternation of silence and sound, e.g., observe behavior for 10 min, play calls for 5 min, observe for 10 more min. Record perch hops, tendency to pick up food (provided), i.e., something simple. Then, possibly, take a quick blood sample??

Interpretation. Would love to discuss. We already know that T-males come less often or less predictably to the nest, but when they get there, they apparently encounter young that beg more. Nevertheless they wait a long time to come again, Why is this? Suppose in captivity they are equally disturbed by hearing begging calls from young, both behaviorally and physiologically, what would that mean? I think it would mean that in the absence of competing stimuli, T-males respond like C-males that cues that ordinarily stimulate feeding. Their failure to come to the nest as often would then be attributed to the greater appeal of stimuli that compete for their attention.

III. Unmanipulated juncos - co-variation in T across stages of reproduction (ask Van Roo)

IV. Unmanipulated juncos - effect of antibody to prl on male parental behavior (ask Schoech)

V. Solitary vireos (ask Van Roo)

Make a start using the T-implant approach with a passerine bird in which males incubate, Solitary Vireos. Implant 5 males as T, 5 as C, and do nest watches to determine whether T interferes with incubation and sample nestlings to see whether rate of EPFs is affected on second broods in males implanted on first brood.

VI. Mate choice according to subspecies (ask Jones)

Ask whether females of two subspecies of juncos (*Junco hyemalis hyemalis* and *Junco hyemalis carolinensis*) prefer males of their own or the other subspecies. If they prefer males of their own subspecies, this may mean that juncos are reproductively isolated at the level of subspecies, at least in captivity. If they were to prefer males of the other subspecies, particularly if northern females prefer Carolina males, this might mean that sexual selection can repress reproductive isolation and retard speciation.

VII. Demography

return rates of nestlings, males, females, mate-fidelity

VIII. Miscellaneous projects

A. Sperm counts and copulatory behavior

Do sperm reserves refill more rapidly in captive T and C-juncos? Are T-males more likely than C-males to mount a stuffed female in the field (??).

In order to determine whether males are likely to be sperm limited, we need to know their 'refilling rate' in captivity and in the wild. This could be accomplished by milking a set of captives at 24 hour intervals and by capturing males at their nests on successive days. We tried this in 96 on captives with limited success, and I am not really sure why except to say that the captives did not respond well to repeated efforts to sample sperm.

We would also like to know whether T affects mounting behavior in the field. If T-males copulate more frequently in the field, we might expect them to copulate more readily with a stuffed female mounted in a precopulatory display and placed on their territories.

If we found an effect of testosterone, I would be interested in the future in trying to take this apart by designing an experiment to distinguish the effects of straight T, an estrogen implant, or an aromatase blocker.

B. Testosterone and flexibility in behavior

Background: Based on our proposal to the NSF, we committed to investigating environmental control of flexible trade-offs between mating effort and parental effort and the

coordinated physiological and behavioral changes involved. We also promised to determine whether fixed hormone profiles (i.e., T-implants) limit male flexibility in a maladaptive way.

The approach will be to create environmental opportunities that should tilt the balance of the fitness equation towards either mating effort or parental effort and then to compare the responses of T- and C-males. One prediction is that as the potential gains from parental effort increase, male physiology should reflect readiness to behave parentally. Alternatively, the greater the potential gains from mating effort, the more male physiology should represent readiness to mate or evidence of having mated (Henrik Smith on starlings).

The manipulations will be brood enhancement or diminishment and induced fertility in females, either mates or neighbors. Dependent variables are to be song, and feeding rates (including time structuring), T and prl, and possibly sperm or responsiveness to predators. We will also look at self-maintenance, i.e., self-protection, grooming, and self-feeding (foraging) vs. any form of reproductive effort.

Predictions. If brood size is enhanced (e.g., 2 vs. 6), males will sing less, feed young more, preen more, possibly eat more themselves, be more likely to detect a predator, pay less attention to an intruder or a females in a pre-copulatory display, have more sperm (because they are not copulating?), turn down their T, have more prl (?), etc. If brood size is reduced, all predictions reversed. T-males less flexible than C-males.

If a neighboring female becomes fertile, predict that all males will feed less and raise T, but changes will be greater in T-males than in C-males.