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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 95:

In 1995, we will continue to examine phenotypic effects of testosterone in male juncos and attempt to relate these to fitness. Objectives for this year include comparisons of T-males and C-males for (1) parental behavior at the nest, including load size, begging vocalizations, and time-structuring, (2) sperm counts on successive days in captives, (3) mounting behavior in captives to compare readiness to copulate and ejaculate size, (4) presence or absence of coccideal oocysts in the feces (4) corticosteroid response to handling stress, (5) cellular components of blood, and (6) field metabolic rate of females mated to T- and C-males.

General Methods

Implanting: We decided to implant as usual from the station and WVN11 southward, creating the usual mix of T- and C-implants. As usual, treatment is being assigned at random to young adults, after blocking for location. Among old adults, unbanded males are not treated because they will settle off the study area. Banded adults that were not treated last year, but that were caught during the summer, are being treated in hopes that we will find their nests this year. Their treatment is being assigned as for young males. Returning adults that were treated last year are being given the same treatment as previously. We were not as effective as usual in removing implants in 95, so there are considerably more C-returns that T-returns this year.

North of the station, we are creating an all-T study area. This will consist of only a small number of birds this year, but should tell us whether this is feasible in the future. It will also create a source of T-males for any T- vs. C- comparisons that need to be made this year. As ususal, we will leave the birds on 714 unimplanted to serve either as control-controls or to do short-term manipulations (see next paragraph).

A reviewer of our NSF proposal suggested that we consider doing short-term implants instead of season-long ones, so that the effect of the hormone is controlled and fresh. This has definite appeal and seems likely to work based on nestwatches I have done in which an implant fell out and was replaced. Feeding of nestlings had been greater than expected, and after the implant was replaced, feeding declined. If we don't do this in 1995, we will do it in 1996.

Overview of objectives

2. Causes of individual variation in sperm reserves.

In order to determine whether males are likely to be sperm limited, we need to know the '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 would also like to know whether T affects mounting behavior, size of the ejaculate, etc., in captives and in the field. If T-males copulate more frequently in the field, we might expect them to mount a captive, estrogenized female after a shorter period of time.

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.

3. Relative investments by males and females in feeding nestlings and the role of T in determining the balance

I am also especially interested in the effect of T on the balance of male and female contribution to parental behavior, and in whether the hormone affects load size, types of foods delivered to young, structuring of time, etc. This is the year I would like to work on these questions, predators permitting. Gigi 's work showed a trend towards greater variance in the intervals between feeding of T-males as opposed to C-males, though there were too few nests to say anything conclusive. I would like eventually to do playbacks in the wild and in captivity, feeding experiments, brood manipulations, etc. But first I think I need to know more about the patterns of feeding and the nature of the vocalizations.

4. **Other hormones**

I am also interested in collaborating with Anne McNabb and Carol Vleck re other hormones, **thyroid and prolactin**; the question simply being how does T affect the levels of other hormones that are important to life and reproduction?

5. **Female mate choice**

These experiments will be conducted by Jennifer Hill under the direction of Dave Enstrom. Jennifer will enhance tail white to see if females prefer males with whiter tails. As a side project, she will also compare song rates of singly and multiply housed males to see if they stimulate or suppress one another's song. She may see whether same-aged males with enhanced white but normal T would be preferred to males with normal white but enhanced T. Problem, I want to leave time for a mounting experiment in the choice aviary!! (see above)

6. Testosterone and male survival (Houtman?): this is potentially a whole set of sub-projects

Do the treatment groups differ in their susceptibility to disease?: mites (inspection), pox (inspection), hematozoa (blood smears), coocidiosis (feces).

Condition: muscle, fat, and hematocrit

Immune status: lymphocytes, kinds and number of wbc

Corticosterone: *cort response to handling* , compare treatment groups, males to females, etc. What is the meaning of Lori's finding that binding proteins differ in the treatment groups. If there is a stress response to handling, the group with lower plasma levels of cort (C-males) might secrete more, whereas those with the higher background levels (T-males) might simply release cort from its bound state. If true, the C-males could show a steeper increase in cort than the T-males.

Behavior: seeking behavioral traits that could be physiologically linked to immune status. This idea comes from Ben Hart; who thinks T might down regulate grooming in impala (more time spent looking for predators or competitors) and that the same might be true for *preening* in birds. Can be done as part of nest-watches.

To test, we could compare males and females for time spent preening or compare portions of the body preened on the premise that preening some areas would interfere with visibility (detecting predators) more than others. Thus, do T-males preen less than C-males or females? Do they preen different parts of their bodies? Does this require captives?

Metabolic rate: *field metabolic rate* as measured using doubly labelled water. The question is how to make the most of this. Get 5 more each of T- and C-males? Try another species? Focus on the female!!!!!!!!

If we think that T-males defend larger territories, then it is possible that females mated to them could increase their feeding rate without greater cost to themselves. This would be because food could be found more readily, without, e.g., having to fly so far/trip for food collection (??).

Fitness measures:

The fitness measure we are most interested in this year is whether males that received T-implants repeatedly show lower return rates than C-males, which is a reason for implanting in the usual way for one more year. We would then meet our promise to measure the effects of asking birds to bear the same treatment two years in a row. We will look for differences in return rates and differences in relative condition of the ones that return. This too is something we promised to do, which means to me we almost have to implant as usual this year.

A fitness angle that might prove fruitful would be to perform short-term manipulations on 714. We would treat neighboring males with T or as controls, then create an opening by removing eggs of one (or both?) of the females, and see who

succeeds in siring young, using DNA techniques to measure paternity. One concern is that the nest would fail before we could determine paternity, but if we could fingerprint embryos that had begun to develop in an incubator, our success rate would be a lot higher.

1996-97 objectives: Coordinated responses to possible investment returns on mating effort and parental effort.

Based on our proposal to the NSF, we are committed to investigating environmental control of flexible trade-offs between mating effort and parental effort and the *coordinated* physiological and behavioral changes involved. I also promised to determine whether fixed hormone profiles (i.e., T-implants) limit male flexibility in a maladaptive way. However, we are not yet ready to do these manipulations, so they will be done in 1996.

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 measure the responses. 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.

The manipulations will be brood enhancement or diminishment and induced fertility in females, either mates or neighbors. Dependent variables are to be song, sperm, T, and feeding rates. Other dependent variables might be time to discovery of a predator near the nest or response to a caged intruder.

We will also look at testosterone and corticosterone to see how they vary in response to opportunities for reproductive success via mating effort and parental effort? We will also look at self-maintenance, i.e., self-protection, grooming, and self-feeding (foraging) vs. any form of reproductive effort?

Procedure: In the field, attempt to measure coordinated changes in expression of traits - find a nesting pair, in some enhance brood size, in others decrease it, observe and quantify song, also quantify preening and feeding of young and foraging (present food), present a chipmunk or an intruder, catch some for sperm, catch others for hormones, especially T, but also prl and possibly thyroid.

Predict that as brood size goes up (2 vs. 6, 1 vs. 5), males will sing less, feed young more, preen more, possilby eat more themsleves, be more likely to detect a predator, pay less attention to an intruder, have more sperm (because they are not copulating?), turn down their T, have more prl (?), etc.

Now if we induce nest failure, so that same male's mate becomes fertile, and were able to measure all the same things, we would predict that the trends would

reverse themselves. However, the behavioral data become much harder to obtain when the male is not tied to a nest. So an alternative would be to make a neighboring female fertile and see how the male's behavior changes at his own nest.

We expect C-males to show plastic behavior but predict that T-males will be less able to respond to enhanced brood size, making a fixed hormone profile disadvantageous.

Miscellaneous projects

I have long, long been interested in **solitary vireos** and the effect of T-implants on their reproductive success. This is because they incubate and I would like to know the degree to which the implants would affect incubation - do one or two birds this year while bleeding a few others to see what natural levels of T are?

I am interested, but less immediately, in **mate choice** between northerns and Carolinas, mate choice as a function of fertility (sperm production), mate choice as a function of female experience. Same for treating indigo buntings with flutamide.