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 96: long working version, May 10, 1996

General Methods

Implanting: We are implanting as usual from the station and WVN11 southward, creating the typical mix of T- and C-implants. Treatment is assigned at random to young adults, after blocking for location. Among old adults, unbanded males are not implanted because they settle off the study area. Returning adults that were treated last year are given the same treatment as last year.

North of the station to the War Spur parking lot is the all-T study area. 714 is being followed by Val. It can be used to generate nests for Steve or possibly males for Sharon and Andy. Steve Schoech will be working with the juncos at the far end of 714 (between 2.3 miles and the bridge).

1. Balance of mating effort and parental effort and reproductive fitness:

(1) Obtain more detailed information regarding parental behavior at the nest, especially (a) any differences in time-structuring and (b) load sizes brought to offspring of T- and C-males (nest watches by whole group).

(a) Time structuring

**Objective:** 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?

Background data: To date we have data collected at nests in 1994 and 1995 on day 6 and day 9: 8 C-males day 6, 9 C-males day 9, 7 T-males day 6, and 10 T-males day 9. Our goal is to have a minimum 12 good watches (normal behavior) at each age and in each treatment. We will compare interfeeding intervals (IFIs) of the two treatment groups.

**Method:** Observe behavior (1.5-2.0 hrs, in the am; try to be done by 10:30 or 11:00) only at nests in which young are day 6 or day 9. This will allow us to combine data with nests observed in 1994 and 1995. It also concentrates effort at nests where feeding is fairly frequent and gets the young just before and just after their voices change from high-pitched wines to raspy calls.

(b) Food brought by adults - load size
**Objective:** to determine whether T affects the nature 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....

(2) Document the link between T- and C-males and offspring by recording vocalizations of nestlings to see how they might differ according to treatment of male (preliminary data available).

(a) Hunger in nestlings: hand-feeding nestlings to satiation and vocalizations

**Objective:** To answer the question of whether the young of T-males are hungrier than young of C-males and also to answer the question of how young communicate their hunger to adults. Finally to determine what motivates females mated to T-males to compensate.

**Method:** Observe nest with day 6 young until young are fed by a parent. Go to nest and set up microphone on edge of nest and remove one nestling from nest. Hold the young in a warm dark place for 60 min while recording. Leave remaining nestlings in place or parents might desert. After 45 min since last fed, return deprived young to nest and remove the others. After the deprived young has settled into nest but before parents have fed it, tap side of nest and induce begging. Record vocalizations by placing microphone on edge of. Do twice with 5 minutes inbetween. Wait five more minutes and feed the young half mealworms to satiation - record how many they eat. Compare treatments.

Alternatively or in addition could standardize visits to nests and note the number of young that beg and the duration of begging in response to a stimulus (tapping). Could use video to increase quality of quantification of response. Could use tape recorder so that it would be possible to compare vocalizations. This would need to be done before fear response sets in or young would crouch or flee.

(b) Are T-males less 'responsive' to begging calls than C-males? - hyperactivity

**Objective:** 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. So what I picture is capturing male at nest in pm, holding him overnight, playing calls to him in 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. Suppose T-males come less often or less predictably, bring bigger loads, encounter young that beg more, yet nevertheless wait along time to come again - but in captivity are equally disturbed - behaviorally and physiologically - by exposure to begging young - what would that mean?

(3) Compare treatment males for flexibility in their feeding behavior and physiology by comparing the effect on both of (a) the creation of a fertile female on a neighboring territory and (b) enhanced or decreased brood size (whole group) (will probably postpone until 1997).

**Background:** 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. We also promised to determine whether fixed hormone profiles (i.e., T-implants) limit male flexibility in a maladaptive way.

First we need to fully characterize the parental behavior of T-and C-males before we impose a manipulation (see 1 and 2). Then 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.

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 rise T, but changes will be greater in T-males than in C-males

Finally, 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. 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) Do sperm reserves refill more rapidly in captive T and C-juncos? Are T-males more likely 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 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.

(5) Compare T and C-males for density of brain receptors for T and prolactin during nestling stage (Steve Schoech).
(6) Analyze return rates of nestlings of T and C-males and of mate fidelity of females mated to males given same treatment over time. (Ellen and Val).

II. Susceptibility to disease and survival:

(1) Compare treatments for presence/absence of coccideal oocysts in feces (Steve Hudman).

(2) Compare treatments for corticosteroid response to handling stress (whole group, Schoech).

(3) Compare treatments for cellular components of blood, e.g., are lymphocytes more common in T-males suggesting an activated immune system or are they less common suggesting that they are disease-free or have exhausted their immunity (early season slides already collected, ??).

The questions: Do het/lymph ratios provide a measure of level of activation of the immune system in juncos, and are they influenced by treatment with testosterone? Do healthy birds have higher ratios or lower ratios? What are the ratios when a bird is battling a disease; when a bird is well prepared to combat disease; and when a bird is recovering from a disease?

Background (i.e., things already known that would lead us to expect one thing or another).

(a). In chickens, birds that are sick or stressed have higher het/lymph ratios (Gross and Siegel). Once they are better - or they have acclimated to the stressor - the ratios return to normal.

(b). Jungle fowl treated with testosterone had higher lymphocyte counts than controls (true?, Zuk).

(c). Coccidiosis is a disease that juncos get (personal observations, Steve's observations).

(d). Juncos treated with testosterone have higher corticosterone than controls.

(e). There is a literature that suggests that prolonged exposure to stress (and thus to corticosterone) can suppress immune function.

(f). Chickens treated with corticosterone are more susceptible to coccidiosis than controls (Gross).
(g). There is a literature that suggests that testosterone, either directly or indirectly, may increase susceptibility to disease, possibly by suppressing immune function (Zuk, Folstad and Karter, etc.).

(h). There is a literature that suggests that secondary sex characteristics that are T-dependent will be more highly developed in healthy individuals. Females might prefer them because individuals that can remain healthy in the face of constant exposure to disease organisms must have 'good genes' that allow them to withstand disease.

**Things we don't know:**

(a). Do coccidial infections influence het/lymph ratios in juncos? If there is a change in the ratio, is it part of an immune response that is mounted against the parasite, or does it represent exhaustion of lymphocytes after a response has been mounted?

(b). Does the increase in corticosterone that accompanies treatment with testosterone affect het/lymph ratios, either in the short run or in the long-run?

(c). Are juncos treated with testosterone more susceptible to disease and is this reflected in their het/lymph ratios.

(d). Compare return rates of T- and C-males treated over time (Ellen and Val)

### III. Solitary vireos

(1) 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.