

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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**MLBS 2003
Primary Goals and Background
April 14, 2003**

GOALS

- 1. Natural variation in hormone response variables (HRV), does relatedness matter?**

To measure natural variation in HRV (individual, seasonal)

- Response to GnRH challenge
- Stress series ("basal," 15', 30') (60'?)
- CBG
- Egg steroids (Egg 3)

To measure repeatability and co-variation among HRV

To measure impact of relatedness on HRV, in particular whether parents/offspring, brothers and sisters, resemble one another more than randomly selected pairs of individuals, also effect of other measures of relatedness using microsats

- 2. Relationship of HRV to phenotype [and someday to fitness in free-living birds]**

To measure sex, age, body size (wing/tail/tarsus), condition, behavior (song, parental behavior), and plumage (tail white) in relation to HRV.

[To relate GnRH to immune function in captives? up to Devin]

[To relate HRV to measures of fitness, e.g., growth rate of young, number fledging, recapture rates, if something there may emerge as we collect our usual data.]

- 3. To compare species for sex difference in response to GnRH challenge, e.g., red-eyed and blue-headed vireos, robins, chipping sparrows, cardinals. [We have permission to do this, so if the opportunity arises, might pursue.]**

BACKGROUND ON NATURAL VARIATION

Many studies have addressed the impact of experimentally elevated testosterone on the behavior and physiology of male songbirds living in the temperate-zone. In general, results are thought to indicate that elevated testosterone is associated with enhanced mating effort, reduced parental effort, and reduced immune function/survival. Some studies have concluded that selection ought to favor males with elevated testosterone; others have concluded the reverse.

Importantly, very few studies have characterized natural variation in hormones or addressed whether endogenous hormone levels co-vary with the phenotypic characters associated with mating effort, parental effort, and survival, and none we know of have been conducted in the field on natural populations. Thus we can rarely say whether individual males that sing more frequently or feed offspring less frequently have higher levels of testosterone or whether males with naturally higher levels of testosterone have less robust responses to immune challenges (but see Duffy et al.).

We also know very little about selection on hormone-mediated traits. Traits that respond to hormone implants are clearly hormone-mediated, and we would predict that selection on one such hormone-mediated trait might lead to selection on the underlying hormone levels, and thus indirectly, to selection on other traits dependent on the same hormone. But in order for traits and hormone levels to respond to selection, they need to be heritable, and in order for them to respond to selection as a unit they need to be genetically correlated. Outside of agricultural settings in which selection experiments have been performed, we have very little information to bring to bear on the question.

We also have very little information about correlations between the sexes, e.g., whether sisters or daughters of males with naturally high levels of testosterone also have high levels. Theory would predict correlated responses to selection in females when natural or sexual selection acts on hormone-mediated traits in males, but we know very little about the correlated expression of hormone-mediated traits in males and females or the magnitude of genetic correlations.

There are at least four important reasons for the absence of information. One is the plasticity that characterizes circulating levels of hormones. Levels of testosterone, for example, vary with time of day (diurnal rhythm, pulsatile release of gonadotropin releasing hormones and gonadotropins), from day to day, with stage of reproduction, and across the breeding season. Levels of plasma corticosterone also reflect a pulsatile release, a daily rhythm, and recent encounters with stressors. Corticosterone also responds immediately to the stress of handling, so is altered simply by measuring it. As a consequence of the lability of plasma hormone measures, any effort to determine whether hormone levels are repeatable, much less heritable, is challenging.

Comparisons must be made of samples collected under similar circumstances, and values need to be corrected statistically for any effects of time of day, time of year, recent encounters with conspecifics, etc. Few study systems have made this feasible, especially in the wild. [An aside, all this variation is biologically interesting, not noise, but it makes is

A second reason we lack sound information about individual variation in hormone levels has been a lack of precision in tritium-based RIAs. These assays are characterized by coefficients of variation that frequently exceed 10%. While this variability has not

interfered with many elegant efforts to compare species or even stages of reproduction within a species, it has proven a serious impediment to studies of individual variation. A third reason for lack of information about individual variation in hormone levels is the concern that the important variation in hormone-mediated traits may not be in the level of the signal (plasma hormone concentrations), but in individual responsiveness to the hormone (e.g., variation in receptor density). Hence some might question whether the effort required to measure the impact of relatedness of hormone levels is justifiable, because, at best, the answer to be gained can provide only a partial answer to the question of why individuals vary in hormone-mediated traits. A fourth pair of factors slowing progress in this area with respect to songbirds is the difficulty of breeding songbirds in captivity and, until recently, of assessing relatedness in the wild. Songbirds tend to disperse from their natal sites, and females tend to disperse longer distances than males, so provide few opportunities to study the impact of relatedness of hormones and hormone-mediated traits based on color-banded populations. Optimistically, use of microsatellite markers to assess relatedness may help to overcome this limitation in wild populations. Despite the difficulties, interest in the evolution of hormone-mediated traits continues to grow, and so consequently does the motivation to find suitable ways to measure individual variation in hormone titers as well as heritability and genetic correlations between the sexes.

One approach holds promise is that of challenging the endocrine system in the way that resembles how immunologists challenge the immune system with antigens in order to assess immune function. Schoech and Wingfield, for example, wished to assess the state of the gonads of non-reproductive scrub jays that cooperated in the rearing of young. Were their gonads competent in the sense that they could respond to an injection of gonadotropin-releasing hormone with an increase in testosterone secretion? If yes, then they could conclude, as they did, that delayed breeding in this species is not attributable to reproductive immaturity, but to some other factor, e.g. social stress or lack of access to proper environmental stimuli.

Our lab (Joe Casto) has recently taken two steps towards the goal of assessing individual variation in plasma levels of testosterone. First, in an effort to reduce variability, we have begun to challenge male juncos with a single intramuscular injection of GnRH and then to measure the resultant increase in circulating levels of T. We hope that any change in T after a challenge will estimate of the capacity of the system to respond to its environment and prove a better estimate of testosterone potential (availability) than do circulating levels. Second, we have turned to enzyme immunoabsorbant assays (EIAs) in place of tritium-based RIAs so as to be able to collect two blood samples in quick succession (one pre-challenge and one post-challenge) without risking harm to the bird. Testosterone RIAs typically require 100 ul of plasma, whereas EIAs can make the same assessment using as little as 20 ul. Collecting 100ul of plasma twice within an hour from a small bird would not be advisable, but collecting 20-50 ul twice poses no risk.

Thus far, based on a sample of 5 birds, each bled ~3 times, we have found that a single dose of GnRH (1.25-2.5 ugGnRH/50 ul PBS injected in the pectoral muscle) induces a 2.5 fold increase in plasma T after 30 min (mean T prior to injection, 1.8 ng/ml; mean after injection, 4.63 ng/ml), and that one hour after injection, levels are back to what they were prior to the challenge. Twice the dose (two injections of 1.25 ug/50 ul PBS) had a similar effect on T, which suggests that the single dose is sufficient to elicit a maximum response. A second assay will allow us to say whether levels were higher earlier than 30

min or returned to pre-challenge levels prior to one hour. Unfortunately, the dose we used did not induce an increase in female T and we are not sure whether that relates to dose, timing, or the state of the females (still relatively short-days when bled). We have much to learn about how response to challenge varies with sex, age, time of day, stage of gonadal development, or any of the other variables known to affect unchallenged levels of T.

The second form of HRV involving a challenge is the 'stress response' in which individuals are exposed to handling stress and bled several times to determine the rate of increase in corticosterone during several serial bleedings. This measure is already in wide use, but most effort to date has compared species or birds at different seasons. We have data from juncos to show that individuals vary widely and consistently in their stress responses and we would like to learn more about this kind of variation.

In sum, among the primary goals for this summer's research are 1) measurements of the response to a GnRH challenge in males, females, and offspring in order 2) to see how consistent the response is and how it varies with relatedness. To this end, we will also hand-rear juncos and bring them back to Bloomington for future measurements and to join our breeding colony. We will also 3) collect eggs for steroid concentrations, and 4) measure CBG and the stress response and relate these HRV to the GnRH challenge.