

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 2006
May 18, 2006
final

Objectives

Hormones are of special interest because they play such an important role in reproduction and influence so many attributes of vertebrate animals. The goal of our research is to explore the role of testosterone in the expression and evolution of complex behavior and physiology in a songbird, the dark-eyed junco. We employ a mix of experimental and correlational approaches. For many years we focused only on males, but we are now studying both males and females. We seek to understand the mechanisms underlying sexual dimorphism and co-variation among phenotypic characters. We are also quite interested in the indirect effects that the hormonal state of parents has on the phenotypic development of their offspring.

Research conducted at MLBS in 2006 will address the impact of experimentally elevated plasma testosterone (T) on adult female dark-eyed juncos, including 1) their parental behavior, 2) their fitness (survival, fecundity, nest success, and extra-pair fertilizations), and 3) the immune status of their offspring.

In addition there will be related side projects. One such project will address individual variation in male aggressive responses to simulated territorial intrusions and the hormonal basis of that variation. A related project may address variation in male courtship responses to the appearance of an apparently fertile female on his territory to determine whether males that respond strongly to males also respond strongly to females and whether those responses can be predicted by testosterone. Another related side project will address the role of neuropeptides in the release of reproductive hormones and behavior.

Finally, there will be smaller projects intended to collect preliminary data that may serve as a basis for future research, e.g., impact of estradiol implants of circulating E_2 , seasonal profile of E_2 .

Background

In the past (1987-2000), we saturated the study area with T- and C-males, observed behavior and physiology, and measured relative reproductive success and survival of males of both types. The task required that we census twice a year, map territories, find nests, bleed/band/weigh nestlings, and then remove implants at the end of summer and mark the years' new juveniles. Numerous sub-projects allowed us to measure the effects of the implants on behavior and physiology. These findings are summarized in Ketterson and Nolan 1992, 1999, Ketterson et al. 2001, Reed et al. 2006).

Beginning in 2001 and continuing in 2002, we turned to implanting females and measuring the effect of T on them. The rationale was to determine the extent to which males and females resemble one another in how they are affected by T. We argued that traits that are unaffected by T in females are insensitive, allowing selection to proceed on these traits in males without accompanying correlated phenotypic responses in females. For traits in which females are sensitive to T, then to the extent that the sexes are genetically correlated, a phenotypic response in females would be expected. The evolutionary implications would depend upon whether the

responses were beneficial or detrimental. These ideas and findings to date are presented in Clotfelter et al. 2004, Ketterson et al. 2005, Zysling et al. in press.

In 2003-2004, we took a break from implanting and focused on natural variation in T and co-variation between T and phenotypic characters including plumage coloration, body size, parental behavior, and immunoglobulins. Our measures were baseline T and T in response to a GnRH challenge, which results in an increase in T (GnRH → LH → T) and varies from individual to individual. We found sex and seasonal differences in response to GnRH (Jawor et al. submitted), co-variation between response to GnRH, response to an STI, and plumage (McGlothlin et al, submitted), and co-variation between baseline T, body size, and a measure of innate immune function (IgG, Greives et al. submitted).

In 2005 we return to implanting females and the effect of experimentally elevated T (EET) on phenotype and fitness and we continue in that vein this year.

Responsibility and credit

We have an MO that has been very successful in the past and serves as a template. Each year the team as a whole collaborates to pursue our joint objectives. This year's projects, for example, build on the implanting of females and the pursuit of their nesting attempts, so everyone, regardless of later credit in the form of papers, will be engaged. A standard example is finding nests. No nest, no study, so we all need to help find them; the same for recording and entering data. In addition, certain individuals have responsibility for particular goals, where responsibility consists of writing proposals and protocols and later analyzing data and writing the first draft of papers that result. This requires cooperation with other members of the team to be sure that studies don't interfere with one another.

Not every paper has every participant as an author, because some of the effort is seen as reciprocal. Typically graduate students and post-docs are first authors on papers that result from projects they conduct. Collaborating REU students are typically authors on those projects as well. Field assistants do not typically earn authorship in the first year at MLBS, but if they participate in multiple years they often do. Because some studies are conducted over multiple years, credit is sometimes shared with earlier participants.

Some papers are not associated with one individual or may summarize many studies, and frequently I am the first author on those. I often co-author with Val. Eric has special status. He has been with us since 1993, and he is our leader in the field, helping to see that everyone succeeds by coordinating the project as a whole. He also summarizes the demographic data from year to year and is typically an author on papers that summarize multiple years of data and that report demography.

Major goals for 2006 (chronological order and primary grad student and REU responsibility)

- 1. Does having been implanted in 2005 influence likelihood of female survival or site fidelity in 2006 (Team)**

2. **Do males that show strong responses to GnRH also show strong response to simulated territorial intrusions? (Joel + his team)**
3. **What is the role of habitat and juxtaposition to other nests in determining variation in various measures of performance and fitness and in the rate of EPFs (Eric)**
4. **Does experimentally elevated testosterone (EET) enhance female aggressive response towards male intruder at the nest (SNI) (Dawn + Dustin)**
5. **Does experimentally elevated testosterone (EET) suppress parental behavior towards nestlings? (Dawn + Dustin + Team)**
6. **Does EET alter the female fecundity, rate of extra-pair fertilizations (EPFs)? (Nicki + Team)**
7. **Does EET affect immune status in developing nestlings (Nicki + Krystle)**
8. **What is the season-long profile of estradiol (Nicki + Team)?**
9. **Does the neuropeptide kisspeptin vary seasonally in its effect on reproductive behavior and hormones? (Sara + Becky)**

Objectives in chronological order

1. **Census population:** Determine which of females implanted in 2005 returned in 2006.
2. **Implant females** by catching them at random at traditional net and trap sites, or off the nest. Measure and mark any males caught in the process. Bleed all adults at capture for DNA and hormones. Measure response to GnRH in males (see Process 2006)(begun 8 April by Joel, Amanda, Jodie, Nicki, thereafter Team). *Phase I ended 13 May 2006, hereafter implant females captured at the nest. Once a female has served for nestling care, she can have her eggs removed on subsequent nesting attempt after she has been implanted.*
3. **Map study area; obtain an accurate description of all nest locations.** Territory boundaries are key to standardizing response to STIs (see below). Territory locations permit us to determine whether females returning in 2006 are faithful to mate or site of 2005 (Joel + Team).
5. **Map nest locations from earlier years.** The goal here is to assess the role of habitat and juxtaposition to other nests in determining variation in various measures of performance and fitness, in particular the frequency of EPFs. First step is to use GPS units to map nest locations since 1992 (Eric).
6. **Relationship between male response to GnRH challenge and male aggressive response to intruder.** This is in completion of studies begun in 2005 in which Joel et al. found co-variation between male T after GnRH, tail white, and increase in T in response to STI. This year the goal is to relate intensity of aggressive behavior to earlier measure of T in response to GnRH (Joel, Amanda, Carrington).
7. **T and female phenotype: effectiveness of implants.** Bleed females before upon implant and afterwards as the opportunity arises, with up to three bleeds per female, with

at least a week between bleeds. Bleeds will reveal effectiveness of implants and permit us also to assess seasonal profile in related hormone estradiol (Nicki + Team).

8. **T and female phenotype: impact on brood patch development/nest-building/egg laying/gaps between eggs/clutch size.** Note brood patch development at each encounter. Obtain more data on whether testosterone interferes with nest-building or egg laying simply by following females during this stage and comparing treatments for measures like time to complete nest, time between completion of nest and appearance of first egg, gaps between eggs, failure to lay, etc. (Data very important, does not call for a special protocol, simply follow the usual procedures, see Eric and Nest 2006)(Team).
9. **[T and female phenotype: egg steroids.** In previous years we have collected an egg from each clutch to determine egg steroids and relate to offspring development. This year we will NOT remove eggs for this purpose and rather have full clutches in order to increase the likelihood that we will see an effect of female T on parental behavior or nestling immune development.]
10. **T and female fitness: rates of EPFs.** Determine whether T affects frequency of EPFs and fecundity (usual techniques, measuring number and quality of offspring, bleeding males, females, newly hatched young for paternity and relatedness)(see nest 2005)(Nicki, Eric, and team).
11. **T and female phenotype: female aggressive response to male intruder during incubation.** Present lure to incubating females and quantify female behavior. (Dustin + Dawn).
12. **T and female phenotype: allocation to parental effort** when rearing nestlings. Measure female brooding rate early in the nestling period (film), feeding rates first without the male, later with the male so as to assess whether females feed at the same rate and compensate with male is missing (Dawn and Dustin + Team).
13. **Female T and offspring phenotype: immune function in nestlings.** Inject offspring with PHA on day 6, return day 7 to compare swelling in T- and C-nestlings. Shall we obtain additional plasma for comparisons of corticosterone? For IgG and complement as was begun by Dawn in 2005? (Krystle + Nicki).
14. **Other extended phenotypic effects of female T on offspring.** Do offspring of T- and C-females differ in growth, condition, and survival (already underway), sex ratio (already underway)? A reprise of similar studies done on males by Casto and Parker-Renga (Team, Nicki for sex ratio?).
10. **Kisspeptin and other neuropeptides.** Individuals and populations vary in the timing of reproduction and the female appears to be 'in charge.' A key question is what determines time of onset of breeding and the maintenance of reproduction, and does the neuropeptide kisspeptin play a key role? Females and males will be sampled to determine whether they vary individually, seasonally, or from each other in the degree to

which they elevate LH in response to kisspeptin injections (Sara + Becky). Also does a kisspeptin injection influence reproductive behavior in response to a conspecific of the opposite sex? (Becky + Sara).

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Additional project ideas for 2006

15. **Relationship between male response to GnRH challenge and male courtship/sexual response to a simulated visit by a female to his territory.** This is a possible extension of Joel's efforts to assess co-variation among male T in response to a GnRH challenge, tail white, increase in T in response to STI and level of aggression shown in an STI. Does male also increase T in response to a simulated female visit to his territory? Is the increase predictable from his early season response to a GnRH challenge (?? + Joel).
16. **Other extended phenotypic effects of female T on offspring.** Does treatment of the mother influence begging vocalizations produced by young? Are young of T-females quicker to beg, do they beg more loudly, do they eat more when given the opportunity? Similar to work conducted by Kennedy and Bentz (manuscript). Would require observations on young, probably on day 7, would probably preclude measuring parental behavior of female on day 7 in presence of male.

Potential projects carried over from earlier years that have never been selected, perhaps another day or year.

- Predictors of juvenile return. An early study showed no effect of juvenile body mass or wing length or frequency of capture on return rate (Ketterson et al. 1991), but we have never related juvenile plasma components with return/no return. Obtain more plasma hormone samples from juveniles to see whether cort or T or IgG predict which ones return. A good late summer project for an interested person.
- Possibly compare the response (hyperactivity) of recently captured parental males and females to tapes of begging calls, use that as a protocol for assessing effect of implants on parental behavior in captives.
- Measure begging response of hand-reared young to simulated treatment-specific feeding schedules to see how nestlings “learn to beg.” See if this would fit with already collected data comparing T- and C-males for the schedules on which they feed their young.
- Isolate effect of T on parental behavior from effect of T on nestling begging by allowing non-T-implanted adults to feed young hatched from eggs laid by T-treated females and T-implanted adults to feed young hatched from eggs not laid by T-treated females. That is, implant some females before egg laying and some after and remove implants from some and not from others during the nestling stage.
- Measure natural variation in flexibility in response to experimentally altered mating and parental opportunities by comparing hormone levels and behavior at the nest when there is or is not a fertile female nearby (because you have failed the neighboring female’s nest) or before and after broods have been enhanced in size.