GOALS 2005
May 11, 2005

Objectives
Research conducted at MLBS in 2005 will address the impact of experimentally elevated plasma testosterone (T) on adult female dark-eyed juncos, including 1) their behavior and physiology, 2) their fitness (fecundity, nest success, extra-pair fertilizations), and 3) plumage and other development of their offspring. The goal is to understand the mechanisms underlying variation in sexual dimorphism, co-variation among phenotypic characters, and cross-generational phenotypic similarities.

Background
In the past (1987-1988, 1989-2000), 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.

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. in press, Zysling et al. in prep.

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 (Jodie et al.), co-variation between response and plumage (Joel et al.), and co-variation between baseline T, body size, and IgG (Tim et al.).

In 2005 we return to implanting females and the effect of experimentally elevated T (EET) on phenotype and fitness.

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 2005 (chronological order and primary grad student and REU responsibility)

1. **Does EET suppress parental behavior towards nestlings? (Dawn and Katie)**
2. **Does EET alter the female fecundity, rate of extra-pair fertilizations (EPFs)?(Nicki)**
3. **Does EET increase yolk T and does elevated yolk T affect plumage and other development of offspring? (Joel, Jenny)**

Objectives in chronological order

1. **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 (see Process 2005)(begun 15 April by Joel, Jodie, Peter, thereafter Team).

2. **Map study area; obtain an accurate description of all nest locations** (Team).

3. **T and female phenotype: effectiveness of implants, impact on nest-building/egg laying**. Bleed females before and after implant. 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. (no special protocol, usual techniques, see Eric and Nest 2005)(Team).

4. **T and female phenotype: egg steroids.** We know that T-females produce eggs with higher concentrations of T than eggs of C-females, but we want to relate egg T to

plumage development in offspring (still to be decided - shall we mark eggs during laying, collect one per clutch? essential to know when female implanted in relation to when eggs collected)(see Eric and Nest 2005 for protocol)(Joel, Jenny, team).

5. **T and female phenotype: allocation to parental effort** when rearing nestlings – female feeding rates first without the male, later with the male so as to assess whether males compensate for any change in female parental effort. May also measure nest defense or response to predator (see Dawn for protocol, Dawn, Katie, and team).

6. **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)(Eric, Nicki, and team).

7. **Female T and offspring phenotype: plumage development in young of T- and C-females.** Hand-rear offspring from day 6 on, compare tail white in offspring (see Joel for protocol, Joel, Jenny, Annie in the field, everyone on team for rearing offspring)

8. **Other extended phenotypic effects of female T on offspring.** Do offspring of T- and C-females differ in growth and survival (already underway), sex ratio (already underway), immune status (IgG). A reprise of similar studies done on males by Casto, Kennedy and Bentz. (team, Dawn for IgG, Nicki for sex ratio?)

9. **Relationship between male response to GnRH challenge and male response to intruder.** This is an extension of studies from 2003-04 in which Joel et al. found co-variation between male T after GnRH and tail white, suggesting that tail white might communicate aggressiveness. To confirm, will challenge male with GnRH on day 1, measure behavioral response to an intruder on day 2 (Joel, Jenny, Annie).

References

Clotfelter E.D., O'Neal, D.M., Gaudioso, J.M, Casto, JM, Parker-Renga, I.M., Snajdr, E.A., Duffy, D.L., Nolan, V. Jr., and E.D. Ketterson. 2004. Consequences of elevating plasma testosterone in females of a socially monogamous songbird: evidence of constraints on male evolution? *Hormones and Behavior* 46:171-178.

Ketterson, E.D., V. Nolan Jr., and M. Sandell. 200x. Testosterone in females: mediator of adaptive traits, constraint on the evolution of sexual dimorphism, or both? *American Naturalist,* in press.

Zysling, D.A., Greives, T.M.*, Breuner, C., Casto, J.M., Demas, G.E., and E. D. Ketterson. 200x. Behavioral and physiological responses to experimentally elevated testosterone in female dark-eyed juncos (*Junco hyemalis*): implications for the evolution of sexual dimorphism, to be submitted to *Proceedings Royal Society*.

Additional projects for 2005 or projects for 2006. Number 10 most amenable to 2005

10. **T and female phenotype: aggression.** Females are very aggressive towards male intruders near the nest, so half way through incubation period, we could present a male lure and use time to capture as a measure of aggressiveness, then bleed females for T.

11. **T and female phenotype: attractiveness, sexual behavior:** In later years, assess impact of female T on female attractiveness in mate choice trials or sexual behavior in relation to measures of EPFs.

12. **Female T and offspring phenotype.** Comparison of ability of offspring of T and C-females to mount a response to an immune challenge, stress response, begging behavior?

13. **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 of IgG predict which ones return. A good late summer project for an interested person.

14. **Add to Bloomington colony**: in late summer transport newly caught juncos to Bloomington (adults or juveniles) to help maintain the colony of juncos there.

Potential projects carried over from earlier years that have never been selected.

- 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 or before and after broods have been enhanced in size.