GOALS 2000, April 24, 2000

The research conducted at MLBS and in Bloomington is divided into three parts: the effect of testosterone (T) on male behavior, physiology, and fitness (the male project), the effect of T on the behavior and physiology of the individuals that associate with testosterone-treated males (T-males) and controls (C-males) (the extended phenotype project), and, in the context of possible constraints on the evolution of T in males, the effect of T on the behavior and physiology of females (the female project).

Each year we prepare the study area by creating T- and C-males, then monitor the relative reproductive success and survival of males of both types in order to assess annual variation in the impact of testosterone on population structure (age ratio, return rate) and components of fitness (EPF rates, predation rates, survival of adults, mass of nestlings at fledging, etc.). To do this, we implant birds, map territories, find nests, bleed/band/weigh nestlings, enter data into the computer, and help to create the daily list.

We also pursue sub-projects that relate to the objectives outlined above. Some of the sub-projects will lead to publishable papers, and others will lay the groundwork for future studies. In 2000, we will continue to quantify effects of testosterone on male behavior and physiology in relation to fitness. We will also measure aspects of the male's 'extended phenotype' in his neighbors, mate, and offspring. and we will begin to assess the effects of testosterone in females.

We also have a tremendous amount of work to do summarizing already collected data on return rates, mate fidelity, and genetic relatedness. And this is the summer when we need to write a proposal to the NSF describing future work.

Listed here are projects for the summer, not all of which we will be able to pursue. Much will depend on how many birds we implant, whether predators are abundant this year, and the interests of members of the crew, but projects marked with ** are highest priority.

1. Map study area

- To make a junco map of MLBS and possibly to see patterns of distribution of T- and C-males, obtain an accurate reading of the all nest locations using GPS, as well as readings for all trap and net locations (Team).**

2. **T and the male phenotype: effect of T on immune function, allocation to parental and mating effort, song system, aggressiveness, and behavior towards neighboring females with fledglings.**

- To assess effects of T on humoral immune function, compare antibody titers of T- and C-males in response to immunization with PHA and sheep red blood cells (SRBC) (Casto, Parker-Renga).**

- To assess effects of T on aspects of parental behavior, compare T- and C-males for load size and actual feeding behavior at the nest (video) (Clotfelter, Schubert). **

- To assess neuro-anatomical effects of experimental elevation of T, compare song control system in T- and C-males in (Casto, Smulders; involves analysis of already collected data).**

- To assess effect of T on aggressiveness, compare T- and C-males for their behavioral response to a simulated territorial intrusion (STI) (Team). **

- To learn why EPFs are higher in years of low nest predation, particularly for C-males, perform fledgling watches when females are fertile to quantify rates of intrusion by T- and C-males (Team). **

3. Endogenous T in males: effect of relatedness, repeatabilities and individual variation in plasma and fecal T.

- To assess heritability of T and T-mediated traits, begin by collecting plasma T under similar conditions from *any* related males or females found on the study area (father-son, brothers-sisters, etc.). [Can also collect samples from unrelated individuals, so long as a DNA sample has been collected from both to assess relatedness.] (Team). **

- To document repeatability and individual variation, collect plasma *and fecal samples* repeatedly from same males during the same stage of reproduction (Team? May do in Indiana as well or instead). **

4. Extended phenotype: male neighbors

- To assess effect of a male's physiology of that of his neighbors, compare T-levels in control males with and without T-neighbors (Team??, could be done with brief elevations and repeat captures on 714).

5. Extended phenotype: females

- Complete project on effect of male's treatment on movements by females when they are fertile by analyzing already collected data (Neudorf). **

- To assess effect of mate's treatment and greater parental care on female immune function, compare females mated to T- and C-males with respect to cell-mediated (wing-web swelling) and humoral immunity antibody titers to SRBC (Casto, Parker-Renga). **

- To relate male treatment to female's physiological phenotype and egg contents, collect plasma and feces from nest building and laying females to document hormone levels during laying (Team?).

6. Extended phenotype: offspring

- To assess effect of male treatment on offspring phenotype, compare young of T- and C-males with respect to cell-mediated and humoral immunity. For cell-mediated immunity, measure response (wing-web swelling) to a foreign antigen PHA; for humoral immunity, measure ability of females to form antibodies against sheep red blood cells (SRBC) (Casto, Paker-Renga) **

- To assess effect of male treatment on offspring phenotype, compare nestling mouth coloration as an indicator of relative condition and as a possible signal of nestling hunger of T- and C-nestlings (Clotfelter, Schubert). **

- To assess effect of male's treatment on female physiology, compare effect of male's treatment on sex ratio of female's offspring by bleeding nestlings when quite young, measuring survival during the fledgling stage, and measuring rate of return of male and female nestlings (Team, is this worth pursuing??)

- Complete manuscript reporting an earlier project on behavior of fed and deprived nestlings from T- and C-nests (Ketterson). **

7. Constraints: the female project

- To assess how selection for T in males might hypothetically affect female aggressiveness and attractiveness, measure the behavioral responses of females to exogenous T (e.g., how long after treatment do females begin to sing? Do they sing more with bigger dose? Are songs of similar structure as male long range song? Are T-females more aggressive and do they consistently obtain higher ranks in flocks?). As a pilot project, begin to assess whether T interrupts incubation by free-living females or alters the behavior of the mates towards females)(Neudorf, McGlothlin). **

8. Heritability, repeatability on captives

- To assess whether we would expect a response to selection on T in males, attempt to measure heritability by rearing young in Bloomington to produce experimental system and opportunity to compare siblings for hormone levels and behavior (Ketterson, Lipar, Wolf, Sumner)**

- Also transport newly caught juncos to Bloomington in late summer to help maintain the colony of juncos there. Possibly practice hand-rearing related (sibling) young for later measurement of T and T-mediated traits. Whether we do this will depend on success of breeding efforts in Bloomington.

9. Demography and data analysis

- To assess effects of T on fitness, continue to compare treatments with respect to reproductive success, analyze new paternity data from Parker lab (Ketterson, Snajdr)**

- To assess effects of T on fitness, make significant progress on analyzing demographic data, 94-present, return rates of adults and young, mate fidelity, fledgling mass and numbers, annual variation in rates of predation, opportunities for EPFs, etc. (Nolan, Ketterson, Snajdr). **

- Keep up with USFWS banding schedules, fitness correlates sheets, and other summary sheets as we go.

10. Possible additional or alternative sub-projects for this or another year.

- Compare treatments for *flexibility* in their response to mating and parental opportunities by comparing their behavior at the nest when there is or is not a fertile female nearby or before and after their broods have been enhanced in size.

- Possibly compare the response (hyperactivity) of recently captured parental males to tapes of begging calls.

- Measure possible impact of male's treatment on female's extended phenotype by comparing degree of hatching (collected some preliminary data in 99, probably not worth pursuing?).

- Relate T to variation in plumage, i.e., tail white. Results to date are negative showing no correlation between previous year's treatment and the amount to white in R4 in feathers grown in the autumn following treatment. Still could look for effects of dominance status, diet, population density, or a dose of T given just before molt.

- Assess importance of early condition to later fitness by obtaining plasma hormone samples from juveniles to see whether cort or T predicts which ones return. A good late summer project.

- 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.

- Document natural co-variation between control levels of plasma T and behavior.

- Does experience with having been mated to a T-male affect whether a female finds T-males more attractive than C-males?

- Compare levels of cortiocosteroid binding globulin (CBG) in free-living T- and C-males by collecting plasma at nest-leaving. To date this comparison has been made only on captives. Consider whether to collect fresh blood at nest-leaving for this purpose.

- Compare treatment males for copulation frequency: do sperm reserves re-fill more rapidly in T- than C-juncos? Are T-males more likely to mount a stuffed female either in captivity or in the field? [compare Schwagmeyer and Mock].

- Quantify comparisons of molt in males whose implants were not removed. Compare pox, condition, body mass, clo pro, fat class, etc. of T- and C-males, i.e., summarize already existing data.