

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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March 2, 1995

All in all, I am anxious to do several achievable experiments that we can finish in a summer. How best to proceed. Proceed by listing things that must be done, things that would be especially enjoyable, and things we might do but that I would find less compelling.

Methodological decisions that must first be made:

key decisions that must be made right away include when to implant, whether to implant, who to implant, how many to hire, who to hire, etc. Do we go for frequency dependence by creating study areas with all T-males and no T-males or differing proportions of T-males, or do we do the usual arrangement of random assignment and the resulting mix of treatments that make the paternity data so difficult to interpret?

Implanting: Several reviewers of our NSF proposal suggested that we bleed when we implant, and implant according to endogenous level of T. Some individuals should get a high dose or a low dose (not possible, because cannot do RIAs until later), or at least so as to know which individuals were in good shape to begin with when we later interpret the data. For example, we might expect the impact of T on performance to be greatest in the individuals that had the lowest T to begin with. [This comes out indirectly in the age comparisons because first-year males have lower T than adults.]

Another reviewer suggested that we consider doing short-term implants instead of season-long ones, so that the effect of the hormone is controlled and fresh. This has definite appeal and seems likely to work based on nestwatches I have done where implant fell out. When we replaced the implant, the feeding rates tracked treatment (high when implant out, low when implant in).

Hiring: Also need to decide how big a team we need, and what skills they need to have, e.g., veterinary skills if doing lab work vs. field skills if doing field experiments.

Permits: Also need to decide whether we will study any species beside juncos, because if so, I need to write animal protocols, and, similarly, if we are going to use isotopes, I need to get permission. Finally, if we need any equipment, I need to buy it now.

Must do:

Based on our proposal to the NSF, I feel obligated to investigate environmental control of flexibility of trade-offs between mating effort and parental effort and the coordinated physiological and behavioral changes involved. I also promised to determine whether fixed hormone profiles (i.e., T-implants) limit male flexibility in a maladaptive way. I promised to create environmental opportunities that would tilt the balance towards mating effort or parental effort and measure the responses. The

manipulations were to be brood manipulations and induced fertility in females.

Thus I proposed to ask how parental effort, including nest defense and feeding young, balances against mating effort, including courtship, mate-guarding, sperm, and aggressiveness. How do testosterone and corticosterone vary in response to opportunities for reproductive success via mating effort and parental effort? How do they affect self-maintenance, i.e., self-protection and self-feeding vs. any form of reproductive effort?

A related issue is one that I am always interested in, which is natural co-variation between hormones and behavior. Based on the literature, it is unlikely that we will find it. To know hormones, we need to capture birds under 'pure conditions,' so a great deal of effort is required and it might be expended simply to show 'no relationship.' Also recall Mike Moore's paper at ASZ about the difficulty of distinguishing co-variation in response from individual variation in threshold level (although the more I think about it, from an evolutionary perspective, it does not matter if the mechanism of variation in plasma levels, i.e., variation in secretion, or variation in response, i.e., variation in threshold required to induce a response).

When looking for natural co-variation between T and muscle condition or fat, or - I hope - sperm, we have more room to maneuver, in the sense that we can measure these things regardless of how we capture our subjects. In other words, we could do a behavioral observation (song rate) and then catch the bird in any way possible to measure its sperm reserves or body condition. Anders Moller, latest TREE, acts as if body condition might be some separate factor affecting the expression of secondary sex characteristics, separate from hormones that is, supporting Anne's inclination to measure body condition using Andy's methods. I am doubtful about the separateness of these things, but Anders gives us a basis for looking.

Most interested in:

I am especially interested in **sperm reserves**. I would like to know (1) the 'refilling rate' in captivity and in the wild, and I would like to know whether T affects mounting behavior, size of the ejaculate, etc. I find this wildly interesting. If we found an effect, 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.

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 - these things have interested me for years, and this is the year I would like to work on them, predators permitting. Gigi was uncovering some trends that interested me. I would like to do playbacks in the wild and in captivity, feeding experiments, etc.

There is Jennifer and the **tail white** experiment. I made a promise on this one

and have to follow through. It would be nice if she could also manipulate some indicator of quality that was responsive to immediate condition, like the color of wattles, but I can't think of a trait like that in juncos (this is a la the article in the January TREE). If we can help Jennifer finish expeditiously, then the chamber will be free to observe copulation rate, response to begging vocalizations, or Jennifer and I could alternate days.

Interested in, but less so:

To a lesser degree, I am interested in possible consequences of testosterone for the immune system. This includes the work like that done by Kim Sullivan's student last summer, Anne's work on hematozoa and white-throated sparrows, cort binding proteins, levels of coccidial infection, etc. Also there is Donna Holmes' work with Steve Austad on aging to consider.

Perhaps the simplest step to add to this story would be to measure **cort response to handling** and then compare treatment groups and also compare the junco to other species. I would like to do this because it might say that whatever the level of binding protein, T-males also respond more (or less?) strongly to stressors. We might want to do this in the early spring, then compare the same individuals caught later after implant.

Another idea comes from Ben Hart - he thinks T-may downregulate grooming in impala (more time spent looking for predators or competitors) and the same might be true for preening in birds? behavioral traits could be linked to immune traits, could compare males and females or the kind of preening - which area of the body, which areas interferes most with visibility? So would T-male preen less than C-male? If find sex differences in preening, tell Ben.

Truth is, I don't know the implication of Lori's finding that binding proteins differ in the treatment groups. If there is a stress response to handling, the group with lower plasma levels of cort (C-males) might secrete more, whereas those with the higher background levels (T-males) might simply release cort from its bound state. If true, the C-males would show a steeper increase in cort than the T-males.

Also to a lesser degree I am interested in **doubly labelled water**. I would like to get something out of what we have done so far but, once again do not want to invest heavily in a 'no difference' comparison. So I would be interested in the barn swallow/junco comparison but don't really see it as feasible this year, and I would be interested, perhaps, in the metabolic cost to females with no male help. But otherwise, it seems labor intensive and chancy. Do we want to simply get 5 more of each male treatment group to add to what we have and simply increase power? If yes, it seems like a job for Zig and Val and me, because we are already invested in it.

If we do another species, or measure relative cost to T- and C-females, Anne might still be interested. If we think that T-males defend larger territories, then it is

possible that females mated to them could increase their feeding rate without greater cost to themselves. This would be because food could be found more readily, without, e.g., having to fly so far/trip for food collection. If we plan to do this, it calls for immediate action on permissions and permits!!!!

I am also interested in collaborating with Anne McNabb and Carol Vleck re other hormones, **thyroid and prolactin**; no particular question, just how does T affect the levels of other hormones that are important to life and reproduction.

I have long, long been interested in **solitary vireos** and the effect of T-implants on their reproductive success. This is because they incubate and I would like to know the degree to which the implants would affect incubation - do one or two birds this year while bleeding a few others to see what natural levels of T are?

I am interested, but less immediately, in **mate choice** between northerns and Carolinas, mate choice as a function of fertility (sperm production), mate choice as a function of female experience. Same for treating indigo buntings with flutamide.

Fitness measures:

In terms of fitness measures, there remains lifespan, which is a reason for implanting in the usual way for one more year. We would then meet our promise to measure the effects of asking birds to bear the same treatment two years in a row. We will look for differences in return rates and differences in relative condition of the ones that return. This too is something we promised to do, which means to me we almost have to implant as usual this year (:-)

Otherwise I am tired of the no-difference story. I am willing to believe that T-males perform less well than controls in the odd year, but I am not sure we would ever have the samples to prove it. For example, we have reason to anticipate another year of high predation coming up, and there is indication that T leads to lower nest success in years of high predation.....so perhaps the effect would be significant this year.....but is the payoff big enough to justify the effort, or more importantly, the things we would not find out if we devoted our time to more fitness data?

The fitness angle that does appeal to me is to take a very controlled approach, which would work best in a year of low predation when that is possible, probably not this year. In that approach, we would treat neighboring males with T or as controls, then create an opening by removing eggs of one (or both?) of the females, and see who succeeds in siring young, using DNA techniques to measure paternity. If we could fingerprint embryos or raise them in an incubator, our success rate would be a lot higher. This seems to ignore the total absence of a documented treatment-related difference, but it would be an effort to find out what happens when all the noise is removed.

Aside: What about the very obvious age difference that Samrrah's EPF data reveal? Why should old males be worse at defending their paternity????? I am really clueless here, but the obvious solutions relate to all the differences we expected from T-males: age differences in mate-guarding, fertility, etc.. Do we need to back up and see if old males respond differently to female fertility, in terms of their hormone profiles, than young males? For example, suppose old males respond to the availability of fertile females, whoever they are mated to, with high T, but young males do not. So young males stay home more and guard more.... fine, but if old males are pressing in, why would they not overcome the young males - perhaps they do, we will know when Samrrah completes the assignments? what if the old males prefer to force themselves on old females (e.g., because clutches are larger), and the old females are more likely to be mated to other old males than to young males, could this explain it? Are old males less fertile than young males so less successful in sperm competition? etc.

I do not really want to start following fledglings again, and I am ready to stop collecting complete sets of blood samples (because the year-to-year variation thing seems unrealistic and there is otherwise no reason for prolonging these measures) and stop weighing and counting nestlings unless it is part of a short-term experiment.

Testosterone, begging, and parent-offspring cooperation

Background: T-males feed their young less often than controls, their mates compensate.

Question: how come? How is the absence of the male perceived by the female, what sustains her greater parental effort, what attribute of the male reduces his feeding rate? Is he less responsive to young? less able to forage? less attracted by food (see ostriches) or is he more attracted by some competing stimulus?

For me the big underlying question is what alterations in male birds occur when they evolve toward or away from offspring care? I want to extrapolate from realistic physiological manipulations performed within a species to the kinds of changes that occur over time when species evolve care or lose it. My focus is passerine birds in which males do or do not feed offspring. Why are some indifferent to offspring and some so heavily involved? In other words, when you look at the loop below, where do the changes take place.

stimulus (begging, visual and auditory) - perception of the stimulus (sensory perception and processing) - internal response to stimulus (hormonal receptiveness to stimulus) - motor response to stimulus (bring food) - modify the stimulus (food satiates young and alters stimulus)

This is a perfectly worthwhile question, but is it manageable enough to permit even a tentative answer?

I am not sure. One way might be to develop some kind of a comparative assay of how males of different species vary in the way they respond to escalated begging calls from young of that same species. Recall that Yasukawa or Whittingham were able to induce feeding in male redwings with really loud playbacks; would that work with other species, would it work with juncos treated with T? If T-males increased feeding in response to playback, what would that mean? Would it mean that their responsiveness to offspring is intact, but the stimuli have to be more intense in order to be effective? In any case, I would be interested in the outcome if we were to play back begging calls to males in a series of species and compare their physiological and behavioral responses. How do males of the more parental species resemble one another and vice-versa for the less parental species?

A secondary big set of questions is behavioral ecological, which is to ask probe the complex of relations between parents and between parents and offspring. How resilient is the arrangement to perturbation, what is the relative degree of cooperation and conflict between the sexes and the generations, does the nature of the within-pair allocation of parental behavior correspond to NT Burley's differential allocation hypothesis, such that unattractive pair members do more work?, is begging an honest signal of need or is it manipulation, etc.

The way testosterone feeds into this set of questions is to upset the balance of PC by tuning down the male's contribution. It is more natural than feather clipping or weights, and less extreme than male removal. One can then look at the response of females and young.....

Sub-questions:

Are nestlings of T-males hungrier than nestlings of C-males?

define hunger operationally as Karen Price did as equal to the number of morsels of food a young will accept (will they be too afraid?), or could tap and offer food and record number of nestlings in a brood that will take food (would work best before eyes open), number

Are nestlings of T-males needier than nestlings of C-male?

define (long-term) need as Price did as condition or deviations from a gender- and age-specific growth curve. could add component of need in energy cost of begging by using 2-label water on nestlings, since need is clearly a function of energy utilization. If offspring that are fed less predictably become more active between feedings because they experienced hunger, would they require more food overall? Is predictability of feeding important for its own sake?

Karen Price wanted to separate hunger and begging so that they could be investigated separately (this is because begging can be equated with hunger only if it is an honest signal, and for some people, that is the question as issue). So Karen, who was interested in within-brood dynamics (unlike me who is interested in between-brood dynamics) defined competitive ability as relative ability to beg. It was an excellent idea to separate readiness to eat from condition and begging intensity, but in the context of between-brood comparisons it is not clear that ability to beg relates to competitive ability more clearly than it relates to need.....hmmm, I am temporarily stuck.

Do T-males organize their time in a way that is more effective for courting females or defending resources - this has to do with absences between bouts of feeding. If male feeds frequently for awhile and then disappears for a long time, is he more likely to be able to fit in a long bout of courting another female?

Are T-males less responsive than C-males either to offspring or to food ?

(1) Do offspring of C- and T-males beg similarly?

For example, are begging calls of T-pairs louder than begging calls of C-pairs? (sound meter, set standard distance, use blind and a scope to see the sound meter, record peak amplitude of begging bout)

If amplitude is similar, then have to ask what keeps females mated to T-males feeding more frequently. Need to measure other attributes of call, e.g., duration of bout, frequency range, peak amplitude (i.e., wavelength of the sound that is loudest)

(2) Is T-male less attracted to offspring stimuli than control male?

Here we could follow idea of Zahavi's post-doc who studied response of members of babbler groups by playback of nestling vocalizations. He would record the young, then, on the same day, playback the calls while 150m from the nest. The adults would respond by picking up food!! and females were more responsive than males, and young more responsive than adults!! We could do similar presentations to males with young. I suppose, if it's a year of very high predation, that we could also do this if males had no young - as an index of stage specific variation in responsiveness, etc.

(3) Are males equally attracted to food items? and an associated question that has long interested me, is load size similar across treatment, and is load size similar across treatment, and

Place feeders near nest, provide mealworms of two size classes (look up notes from last year's AOU re Kim Sullivan's student

(4) Is T-male more attracted to non-offspring stimuli associated with reproduction than C-male?

e.g., if given a choice in Dave's cage of conflicting stimuli such as a female to court or a young to feed (broadcast begging calls), which stimulus would attract the male more?

This whole area is a little foreign to me. I think it must be ethology or motivation. For example, if you assert that T-males are more attracted by stimuli not associated with feeding, such as other males or their females, is that implicitly to say that they are less attracted to young? Or is there a way to examine attraction to stimulus a, attraction to stimulus b, and then to describe behavior as the integration (sum?) of relative changes in attraction to a and b?. In that case, it seems to me that behavior could change if only one stimulus changed in attractiveness. Note also that I am speaking only in terms of attractiveness, not aversion.

Testosterone, copulatory rate and sperm production:

Background: T-males and controls have fewest sperm when their mates are fertile, they have more sperm while their mates are incubating and C-males have more than T-males. Finally, C-males have more sperm yet just before their young fledge, while T-males have no more than they did during incubation.

This pattern is consistent with the size of the sperm reserve being determined by the frequency of copulation, though, of course, differences in production are also possible.

Sperm mixing - work out a study with Elaina and carry out part on juncos and part on white-throated sparrows.

