

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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Sexually Dimorphic Gene Expression in the Nucleus Taeniae (Medial Amygdala) of a Non-Model Songbird

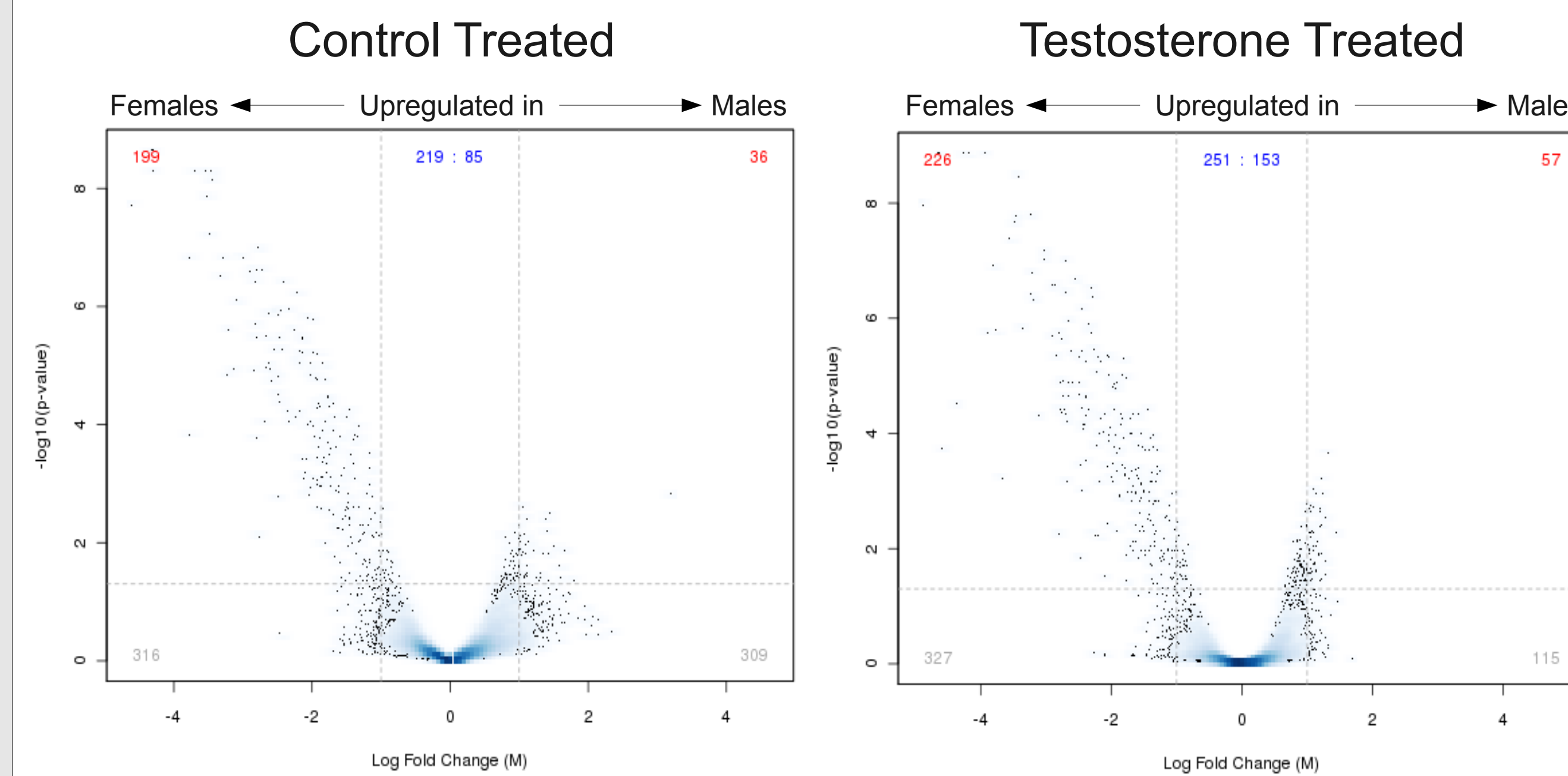
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Introduction

- Males and females share genomes, but they often differ in morphology, physiology, and behavior
- This paradox may be resolved if the sexes differ in gene expression in tissues controlling dimorphic phenotypes
- Testosterone (T) is a steroid hormone that may mediate these sex differences expression because:
 - Circulating levels are higher in males than females
 - Broadly affects gene expression via androgen receptors
 - Mediates individual and sex differences in aggressive and sexual behaviors
- The genomic pathways by which these differences are mediated, however, are not yet fully understood
- We examined gene expression in the Nucleus Taeniae (nT), a brain region, homologous to the medial amygdala¹ known to:
 - Be rich in androgen receptor, predicting aggression^{2,3}
 - Mediate dimorphic sexual and aggressive behavior¹
- The nT is likely to reveal sex differences in gene expression which may be further masculinized by exogenous T

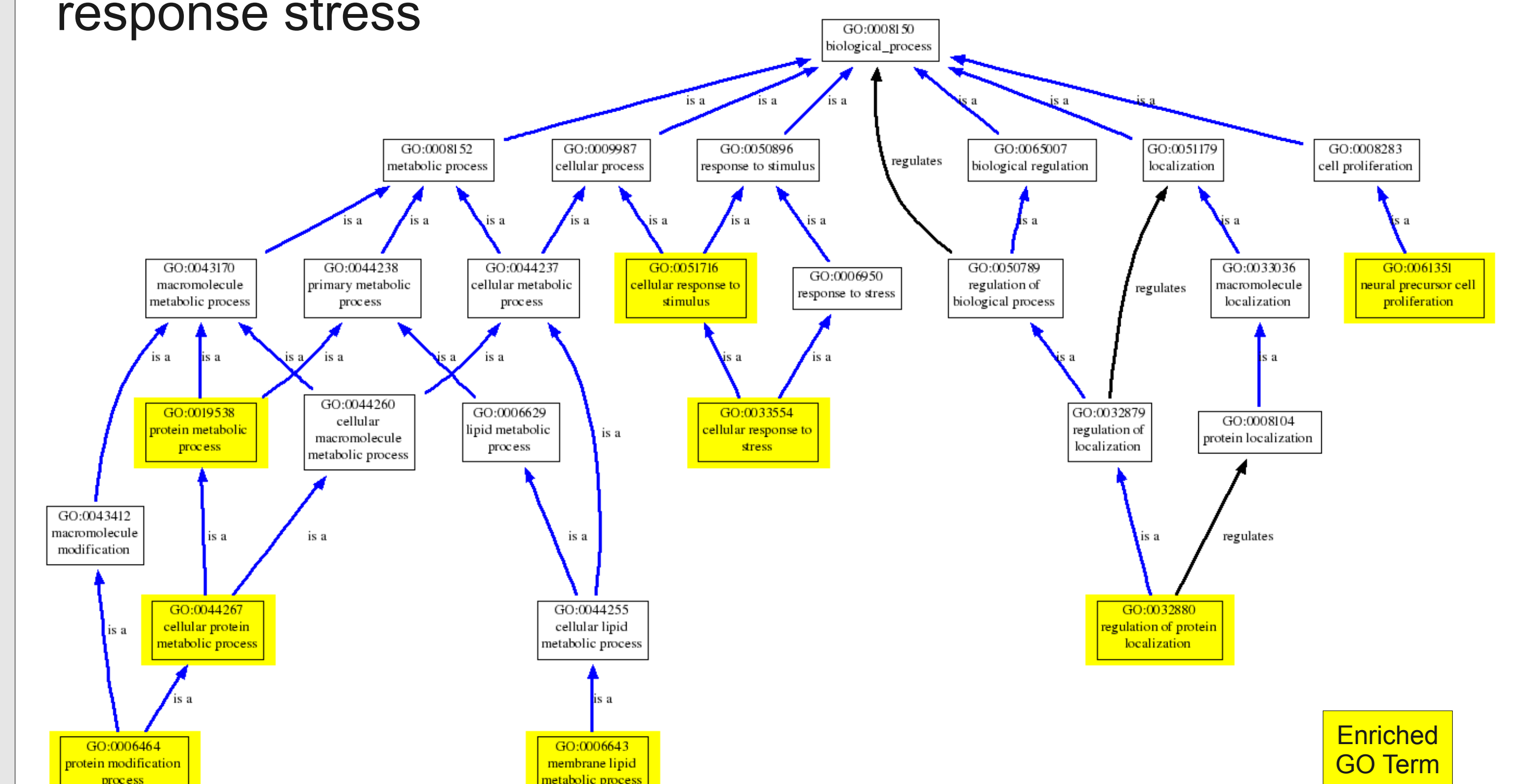
Sex Differences in Gene Expression



- Identified sex differences in nT gene expression in both control and testosterone treated groups
- Most differentially expressed genes overlap, and pooling the treatments yields more statistical power to identify genes

Gene Ontology Analysis

- Sexes differ in the expression of genes regulating protein modification and localization, lipid membrane signaling, and response stress

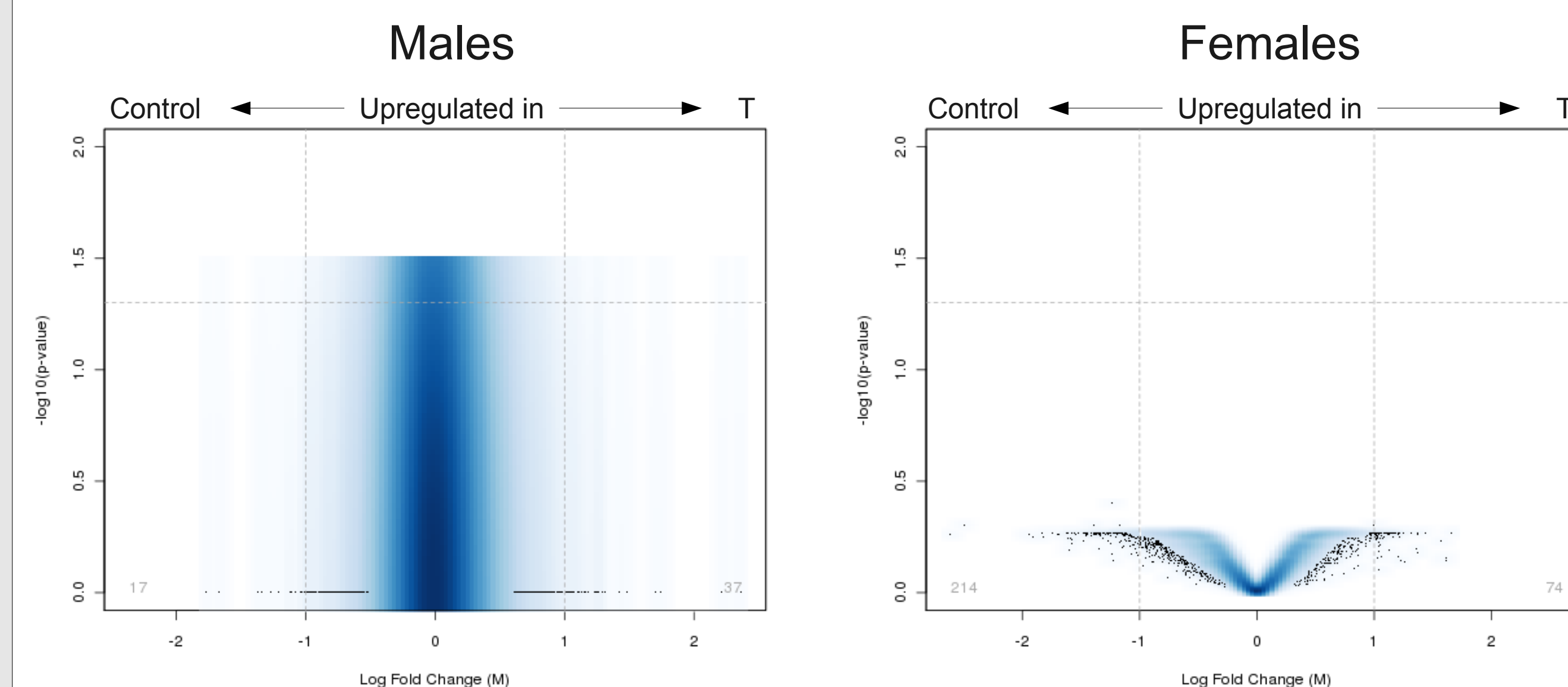


- Pooled the T and C groups to increase statistical power
- Identified 44 GO terms enriched in our data set
- Protein modification includes increased ubiquitination in males
- Membrane lipid process is largely cell-signalling sphingolipids
- Cell response to stress includes:
 - Chromatin cohesion and collagen upregulated in females
 - DNA repair and genome stability upregulated in males
- Protein localization includes transcription factor localization

Questions

- Do males and females differ in neural gene expression?
 - Which genes are involved in these differences in the nT?
- Does exogenous T masculinize neural gene expression?
 - Does T affect males and females in the same manner?

No Significant Effect of Testosterone



- No probes or contigs were significantly different in either sex
- Females had several contigs with strong trends

Dark-eyed Junco

- Seasonally breeding sparrow
- Extensively studied breeding biology⁴
- Many previous T implant studies⁵
 - Both free-living and captive
 - Implants reliably elevate T
 - T affects sexually dimorphic behaviors
- Natural variation in T is correlated with sexually dimorphic physiology and behavior⁴

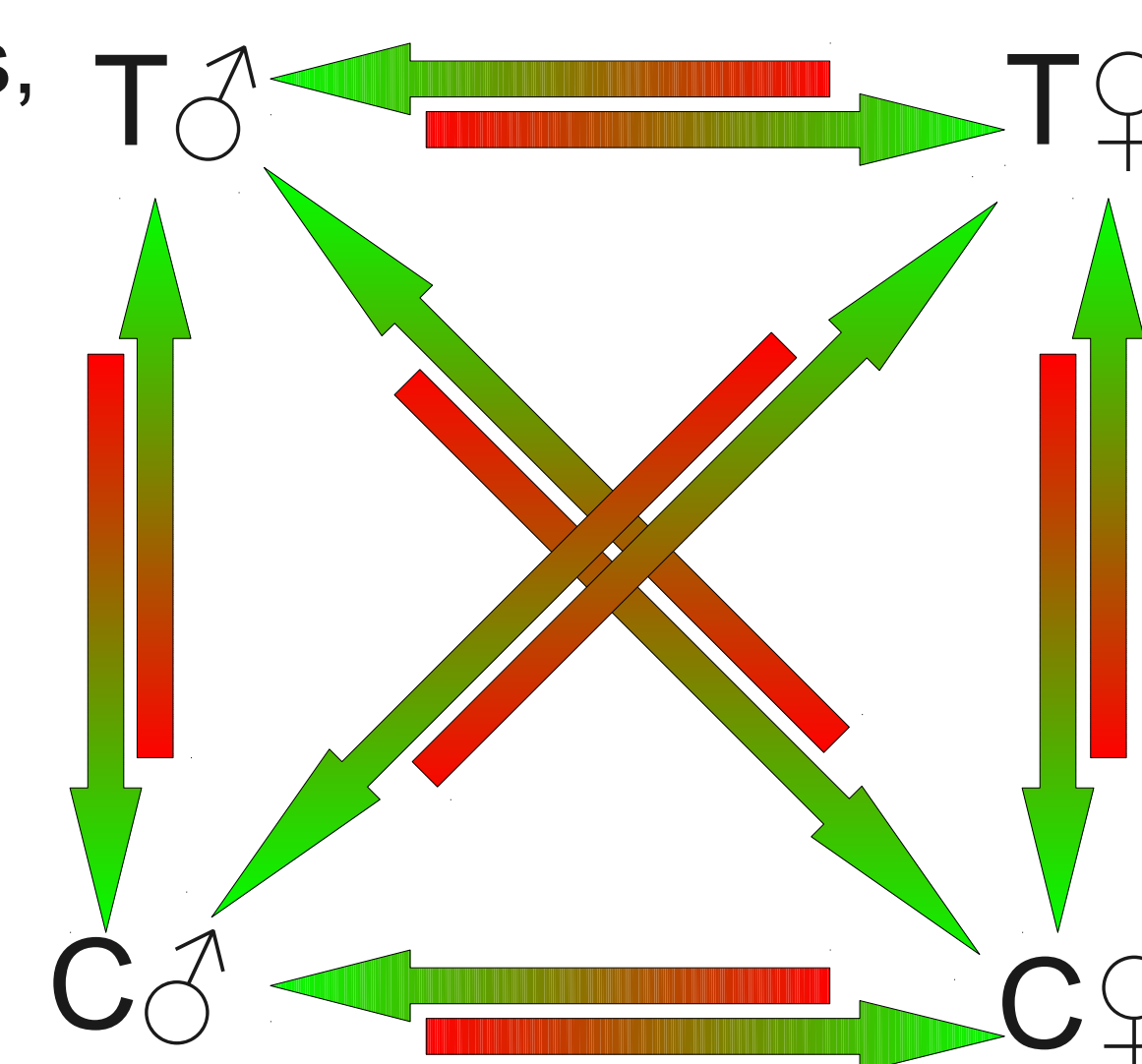


Custom Junco Microarray

- Sequenced transcriptome from RNA of 15 tissues
 - Used one male and one female
 - 1.2 million reads, 433 million bases of sequence
 - Assembled reads into 40,000 contigs, many singletons
 - Annotated with BLAST⁶ and BLAST2GO⁷
- Custom 12-plex Nimblegen microarrays
 - 3 probes from each viable contig
 - 1 probe from each of 34,000 singletons
 - Total of 137,000 features
- Make 12 direct comparisons (24 samples) on a single chip

Experimental Design

- Captured 12 male and 12 female juncos, housed individually in semi-natural outdoor aviary
- Implanted half with T filled implants, half with empty implants
- Collected tissue after three weeks
- Extracted RNA in TRIzol
- Compared gene expression
 - Used 6 biological replicates
 - Made all direct comparisons
- Analyzed using limma⁸ in R
- GO enrichment analysis conducted with BiNGO⁹



Conclusions

- Males and females differ in the modification proteins, cell signaling, and several DNA repair mechanisms in the nT
- Testosterone does not affect gene expression in the nT
 - T effects on behavior may not act directly on the nT
- Adult administration of T is not sufficient to masculinize the nT
 - Role of T is more complicated than previously thought

Future Directions

- Does T affect gene expression in other brain regions?
- Are females responding more strongly to T than males?
- How does natural variation in T affect gene expression?

Acknowledgments

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Literature Cited

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