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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requirements for citing the document.
Title:
Testosterone in Female Songbirds: Natural, Sexual, and Correlated Responses to Selection

Project Participants

Senior Personnel

Name: Ketterson, Ellen

Worked for more than 160 Hours: Yes

Contribution to Project:

Post-doc

Name: Jawor, Jodie

Worked for more than 160 Hours: Yes

Contribution to Project:
Dr. Jawor participated in field work, conducted hormone assays, and was a co-author on manuscripts. She was supported by Indiana University.

Name: Whittaker, Danielle

Worked for more than 160 Hours: No

Contribution to Project:
Lab administrator and person in charge of molecular assessment of relatedness, salary paid by Indiana University; supplies provided by award

Graduate Student

Name: McGlothlin, Joel

Worked for more than 160 Hours: Yes

Contribution to Project:
Mr. McGlothlin conducted field work, analyzed data, and wrote manuscripts. He was supported by an NIH T32 training grant and an NSF DDIG

Name: Gerlach, Nicki

Worked for more than 160 Hours: Yes

Contribution to Project:
Ms Gerlach conducted field work and hormone assays and analyzed data. She was supported by an NSF predoctoral fellowship

Name: O'Neal, Dawn

Worked for more than 160 Hours: Yes

Contribution to Project:
Ms O'Neal conducted field work and analyzed data. She was supported by an NSF predoctoral fellowship

Name: Schrock, Sara

Worked for more than 160 Hours: Yes

Contribution to Project:
Ms Schrock conducted field work and genotyped offspring. She was supported by this award and by Indiana University

Name: Reichard, Dustin

Worked for more than 160 Hours: Yes
Contribution to Project:
Mr Reichard is conducting field work. He was first supported as an REU supported by a site award to Mountain Lake Biological Station. He is now a graduate student at Indiana University.

Name: Atwell, Jonathan
Worked for more than 160 Hours: Yes
Contribution to Project:
Conducting research on juncos in California, NSF GRF

Name: Cain, Kristal
Worked for more than 160 Hours: Yes
Contribution to Project:
Conducting research on juncos in South Dakota and Virginia

Name: Bergeon, Christine
Worked for more than 160 Hours: Yes
Contribution to Project:
Conducting research on juncos in South Dakota

Undergraduate Student
Name: Metitri, Ediri
Worked for more than 160 Hours: Yes
Contribution to Project:
laboratory assistance at Indiana University, molecular relatedness

Technician, Programmer
Name: Snajdr, Eric
Worked for more than 160 Hours: Yes
Contribution to Project:
Mr Snajdr conducted field work, hired field assistants, summarized data, maintained a data base, prepared manuscripts, ordered supplies. He was supported by this award

Other Participant
Name: Bessler, Amanda
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Knox, Carrington
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Dapper, Amy
Worked for more than 160 Hours: Yes
Contribution to Project:
Field assistant in Virginia

Name: Komrey, Sarah
Worked for more than 160 Hours: Yes
Contribution to Project:
conducted field research on juncos in South Dakota
Research Experience for Undergraduates

Name: Ainsworth, Krystle
Worked for more than 160 Hours: Yes
Contribution to Project:
Ms Ainsworth is conducting field work. She is an REU supported by a site award to Mountain Lake Biological Station.

Years of schooling completed: Junior
Home Institution: Same as Research Site
Home Institution if Other: Spelman College
Home Institution Highest Degree Granted (in fields supported by NSF): Master's Degree
Fiscal year(s) REU Participant supported: 2006
REU Funding: REU supplement

Name: Houdek, Rebecca
Worked for more than 160 Hours: Yes
Contribution to Project:
Ms Houdek is conducting field work. She is an REU supported by a site award to Mountain Lake Biological Station.

Years of schooling completed: Junior
Home Institution: Same as Research Site
Home Institution if Other: University of Minnesota
Home Institution Highest Degree Granted (in fields supported by NSF): Doctoral Degree
Fiscal year(s) REU Participant supported: 2006
REU Funding: REU supplement

Name: Young, Rebecca
Worked for more than 160 Hours: Yes
Contribution to Project:
Ms Young is conducting an experiment on captive birds in Bloomington for her honors thesis. She is supported by an REU supplement to this award.

Years of schooling completed: Freshman
Home Institution: Same as Research Site
Home Institution if Other: University of Minnesota
Home Institution Highest Degree Granted (in fields supported by NSF): Doctoral Degree
Fiscal year(s) REU Participant supported: 2006
REU Funding: REU supplement

Name: Spevak, Erin
Worked for more than 160 Hours: Yes
Contribution to Project:
field assistant, independent project on nestling immune function and symmetry in relation to treatment of mother

Years of schooling completed: Sophomore
Home Institution: Same as Research Site
Home Institution if Other: University of Minnesota
Home Institution Highest Degree Granted (in fields supported by NSF): Bachelor's Degree
Fiscal year(s) REU Participant supported: 2007
REU Funding: REU supplement

Name: Schultz, Beth
Worked for more than 160 Hours: Yes
Contribution to Project:
field assistant, independent project on relationship between male T in response to GnRH, male courtship, and male aggression

Years of schooling completed: Sophomore
Home Institution: Same as Research Site

Home Institution if Other:

Home Institution Highest Degree Granted (in fields supported by NSF): Doctoral Degree

Fiscal year(s) REU Participant supported: 2007

REU Funding: REU supplement

Name: Campbell-Johnson, Sam

Worked for more than 160 Hours: Yes

Contribution to Project:
field assistant, will conduct independent project comparing members of an urban and a wild population for their response to potential nest predators

Years of schooling completed: Junior

Home Institution: Other than Research Site

Home Institution if Other: Earlham College

Home Institution Highest Degree Granted (in fields supported by NSF): Bachelor's Degree

Fiscal year(s) REU Participant supported: 2007

REU Funding: REU supplement

Name: Shapiro, Jeff

Worked for more than 160 Hours: Yes

Contribution to Project:
REU student in Virginia

Years of schooling completed: Junior

Home Institution: Other than Research Site

Home Institution if Other: University of Georgia

Home Institution Highest Degree Granted (in fields supported by NSF): Doctoral Degree

Fiscal year(s) REU Participant supported: 2008

REU Funding: REU site award

Organizational Partners

University of Virginia
Research conducted at biological station belonging to the University, Mountain Lake Biological Station

University of California San Diego

Other Collaborators or Contacts

Professor Creagh Breuner, University of Texas, University of Montana
Professor George Bentley, University of California, Berkeley
Professor Gregory Demas, Indiana University
Professor Trevor Price, University of Chicago
Dr. Joseph M. Casto, Illinois State University
Dr. Ethan Clotfelter, Amherst University

Activities and Findings

Research and Education Activities: (See PDF version submitted by PI at the end of the report)

Findings: (See PDF version submitted by PI at the end of the report)
Training and Development:

The students and post-docs listed in the personnel section of this report had opportunities to engage in the following. Not all participants learned all these skills. We attempt, however, to have each student leave the program, however long they are associated with it, with an understanding of the goals of the project, the nature of the methods employed in the measurements and analysis, and for as many as possible, we provide a stint in the field.

1. Conducting field work at Mountain Lake Biological Station (MLBS) where they could observe the behavior of birds in their natural surroundings, interact with peers and faculty researchers on a daily basis in an informal setting, and receive training in experimental design and data analysis. Some were participants in the REU program administered by MLBS that offers research training and more, including practical advice for applying to graduate school and ethical conduct of science.

2. Learning a series of techniques for studying birds in the field, including nest-finding, capturing birds in nets and traps, taking blood samples, entering data accurately, and planning field procedures in a constantly changing field environment. Other related techniques included measurements of body size, image analysis of plumage variation, quantifying the progression of molt.

3. Learning how to care for captive birds so as to keep them healthy and safe from predators. Learning why it is essential to record animal care protocols and the rules associated with their care. Making progress towards a goal of not only maintaining songbirds in captivity but also breeding them.

4. Learning laboratory procedures, including microsatellite analysis of genetic relatedness (DNA extraction, PCR, identifying allelic variation, software to relate genotype to relatedness), how to assay for hormone concentrations in blood.

5. Learning to observe behavior closely and to quantify it by first identifying behaviors and then observing, videotaping, recording vocalizations, or all three.

6. Arising from a collaboration with former post-doctoral student Dr. Joe Casto, now of Illinois State University, we developed the GnRH technique for measuring testosterone responsiveness and assessing seasonal and individual variation in the field. The GnRH challenge is a well established technique but it had not been assessed for its repeatability or predictive power regarding individual variation in plumage or behavior in free-living animals.

7. Owing to the collaboration with IU colleague Greg Demas, many students associated with the research are learning eco-immunological techniques. Earlier we were assessing cell-mediated immunity with PHA as the antigen and humoral immunity with KLH and sheep red blood cells as antigens. Members of the research group are now able to assay for circulating levels of immunoglobulin G (IgG) and innate immunity (complement assay and bactericidal killing assay).

8. Some members of our research group have become expert at quantitative methods for measuring selection and fitness (e.g., animal model, lambda as a measure of population growth, Lande/Arnold selection gradients). All members gained in their ability to assess data graphically and statistically.

9. New methods that lab members have begun to employ over the course of the award include cloning and sequencing functional genes (MHC) and measuring variation in volatile compounds in preen gland secretions (HPLC). The former can be attributed to post-doctoral student Danielle Whittaker; the latter is thanks to a collaboration with IU organic chemist Milos Novotny and his collaborator Helena Soini.

The numbers of people receiving training under this award are listed here.

Contribution to the development of human resources.?Students receiving training with the help of BSC 05-19211: 2 post-doctoral associates (2 female), 10 graduate students (4 male, 6 female, 1 African-American), 16 undergraduate or post-undergraduate students (5 male, 11 female, includes 1 African-American, 10 REUs to this award or to Mountain Lake Biological Station). Of these, 7 have entered graduate school or are in the process of applying, 5 are still undergraduates, and 4 are co-authors on publications or manuscripts.

Post-doctoral associates: J Jawor (now Asst. Prof., Southern Mississippi U), D Whittaker (still in training, SiT). Graduate students: J Atwell (SiT), C Bergeon (SiT), K Cain (SiT), N Gerlach (SiT), T Greives (SiT), B Heidinger (now post-doc, U of Glasgow, Scotland), J McGlothlin (now post-doc, U Virginia), D O?Neal (SiT), D Reichard (SiT), S Schrock (completed M.A.), D. Zysling (Post-doctoral Research Associate,
Indiana University. Undergraduate students and field assistants: K Ainsworth, O Ali, A Bessler, S Campbell-Nelson, A Dapper, B Houdek, C Knox, S Komrey, A Lindsay, K Pavlis, J Phillips, D Reichard, K Robertson, B Schultz, J Shapiro, E Spevak, P Stevens, E Swanger, R Young.

Outreach Activities:
Several of our recent findings have been picked up by the media.


The first author is former post-doc with our group Wendy Reed. Reed is now an Assistant Professor at North Dakota State. Reed et al. analyzed 9 years of data on testosterone and control males and reported higher mortality in T-males but also higher fitness because T males more than compensate for higher mortality with greater mating success.

The study was highlighted by CNN.com and the Washington Post from which it was printed in the Indianapolis Star


It was also labeled a must read paper on the Faculty of 1000 and made a feature in the Alumni Magazine for North Dakota State University.


This paper was featured in Birding Magazine, January-February 2006, p.30. This article by McGlothlin et al. was based the same long-term data set and reports that sexual selection acts on combinations of traits in male juncos, namely body size and a plumage character known as tail white.

Another summary of our work appeared in an Indiana University publication known as Research and Creative Activity. This one focused on human nature and discussed animal behavior research from our group and is posted on line.


It was highlighted with an IU media release


and selected on Am Nat's web page as the highlighted paper for the month of December that produced its own press release
http://reaktionbooks.com/AN/papers.html
and was picked up by various media.

It was also the subject of a zany video that is actually worth watching.

http://www.sciencentral.com/articles/view.php3?type=article&article_id=218393015

Other web postings, none very serious-


http://www.physorg.com/preview111664686.html
During the winter of 2009 students associated with the project made presentations to the local Audubon Society and one of them was picked up by the Bloomington paper, the Herald Times. Here is the story.

Birds: Not just for amateurs, as IU grad students do avian research to help some species survive

Most birders are hobbyists, ranging from casual to obsessive. Then there are the professionals ornithologists who spend their lives in for ... 2/22/2009

Our laboratory website provides access to our research, including mounted pdf version of papers

http://www.indiana.edu/~kettlab/

**Journal Publications**


dark-eyed juncos (Junco hyemalis)", General and Comparative Endocrinology, p. 182, vol. 149, (2006). Published,


testosterone in female dark-eyed juncos (Junco hyemalis carolinensis", Hormones and Behavior, p. 200, vol. 50, (2006). Published,


Soini, H.A. , Schrock, S.E., Bruce, K.E., Wiesler, D., Ketterson, E.D. and M. V. Novotny, "Seasonal variation in volatile compound profiles of preen gland secretion of
the dark eyed junco (Junco hyemalis)", Journal of Chemical Ecology, p. 183, vol. 33, (2007). Published,


McGlothlin, J. W., J. M. Jawor, and E. D. Ketterson, " Natural variation in a testosterone-mediated trade-off between mating effort and parental


Books or Other One-time Publications

Web/Internet Site

URL(s):
http://www.indiana.edu/~kettlab/index.html

Description:
This is our Indiana University lab web site on which my students and I describe our projects and post our publications. I will arrange to have NSF acknowledged.

Contributions within Discipline:

Contributions

Disciplines: Evolutionary biology, Behavioral endocrinology, Animal behavior, Behavioral ecology.

The subject of this research, the dark-eyed junco, provides an unusual opportunity to understand how evolution interacts with hormones to give rise to differences and similarities between the sexes.

Research conducted under this award will uncover the extent of resemblance between the sexes in their phenotypic sensitivity to testosterone, which in turn will predict the potential for both direct and correlated responses to selection. Traits that prove sensitive to an increase in T in only one sex are predicted to evolve independently; traits that prove sensitive in both sexes may respond in correlated fashion. If the sensitive traits are beneficial in one sex and detrimental in the other, they will provide evidence of constraint. Alternatively, the sensitive traits that have
fitness consequences in only one sex but are neutral in the other will indicate sexual independence. Whether or not the sexes are hormonally correlated in natural variation in T and response to GnRH will provide independent insight into the level of evolutionary interdependence between the sexes. Finally, the hormonal mediation of aggression will serve as a case study to explore sexual inter- and independence.

**Contributions to Other Disciplines:**
Other disciplines: Population biology, community ecology, animal development, chemical ecology

Research conducted under this award and those that preceded it have contributed to understanding in population biology (modeling fitness through lambda and relative growth of hormone treated and control populations), community ecology (the impact of acorns and small mammals on temporal variation in fecundity and abundance in the junco), animal development (role of early exposure to hormones in explaining phenotypic variation), and chemical ecology (seasonal variation in preen gland constituents). The contributions to population biology are documented in Reed et al., community ecology via Clotfelter et al; development in Jawor et al. 2007; chemical ecology in Soini et al. 2007.

**Contributions to Human Resource Development:**
Research conducted under this award provided opportunities to train future scientists, some of whom were recruited from Indiana University's REU program in Animal Behavior, which is devoted to members of groups underrepresented in science http://www.indiana.edu/~animal/academics/reu.html

To enumerate this award contributed to the training of the following people.

Associated Postdoctoral Scholars Danielle Whittaker, 2006-present, Lynn Sieffermann, 2006-present, now Asst Professor Appalachian State University, Jodie Jawor, 2002-2006, Southern Mississippi U; Wendy Reed, 2000-2001, North Dakota State U; Joe M. Casto 2000-2003, Illinois State U; Graduate Ph.D. Students- Jonathan Atwell, Christy Bergeon, Kristal Cain, Nicole Gerlach, Joel McGlothlin, Dawn O'Neal, Dustin Reichard,

Research conducted under this award also enhanced the quality of three university classes taught by Ketterson: an undergraduate class in Biology of Birds and two graduate classes, Techniques in Reproductive Diversity and Professional Ethics for the Bio-behavioral Sciences. NSF support has allowed me to bring personal experience to classroom, field and lab exercises, and discussions of ethical issues. Potential societal implications of research supported by this proposal include implications for (1) impact of endocrine disrupting chemicals in the environment, (2) improved methods for breeding captive songbirds for conservation, and (3) a greater understanding of the relationship between sex and gender.

**Contributions to Resources for Research and Education:**
Research conducted with support from this award is being compiled into a readily accessible database that will be made public in time. We are currently beginning to retrievably archive the cumulative results of our research.

**Contributions Beyond Science and Engineering:**

**Conference Proceedings**

**Categories for which nothing is reported:**

Any Book
Any Product
Contributions: To Any Beyond Science and Engineering
Any Conference
MAJOR FINDINGS OF THE PROPOSED RESEARCH (these are presented in relation to the objectives described in the proposal, using the same order as the activities file and the same headings. We are in the first year of the award so while our activities have led to findings, many are still preliminary).

1) To experimentally elevate testosterone (T) in females, measure the phenotypic effects, and compare them to previous results from males.

In a study completed prior to the award (Clotfelter et al. 2004), we had concluded that experimental elevation of T in females did not reduce nest defense, a conclusion that differed from an earlier one regarding males. However the data on females were obtained during incubation, a behavior that males do not perform. When we recently compared T- and C-females for their defense of nestlings, then, as in males, it appears that nest defense behavior is suppressed in females as well. A working graph, reproduced below, shows that females of both treatment defend nestlings more than eggs, but that the increase in defense between stages is less for T-females than for C-females. We are currently collecting more data.

2) To determine fitness consequences of experimentally elevated T in free-living females
Being treated with T significantly reduces the likelihood of a female's reproducing, in part because T-females are less likely to build a nest (19 of 48 T-females, 33 of 53 C-females, p < 0.05), and in part because the rate of nest predation is significantly greater in T-females (daily survival rate, 0.92 in T-females, 0.96 in C-females (O’Neal et al. in prep.). Data
supporting these conclusions are presented below.

Ongoing studies are comparing T- and C-females for survival and mating success.

3) To assess individual variation in hormonal responsiveness and the potential for correlated response to selection.
Response to a standardized injection of gonadotropin releasing hormone (GnRH) is repeatable (Jawor et al, in press). Baseline T prior to GnRH injection co-varies with immune function (levels of IgG and complement, Greives et al. in press), whereas T in response to a GnRH challenge predicts a plumage character (tail white, McGlothlin et al. submitted) and the extent to which T increases naturally after a simulated territorial intrusion (McGlothlin et al., submitted). Figures relating these three findings are reproduced here.
Collectively these results are truly important. First they validate earlier experimental results that revealed relationships among immune function, aggression, and T in male juncos. But they go farther in showing that natural endogenous levels of T co-vary positively with aggression and negatively with immune function. Thus they provide support for hormonal mediation of a trade-off between traits that are related to reproduction (mating success) and survival. The implication is that individuals living in situations that called for frequent elevations of T in response to social cues would have reduced ability to fight off infections.

These findings also complement earlier experimental results, which showed that females prefer males with higher T and whiter tails and that when these traits are presented in opposition, females are split in their preferences. The current data show that tail white and T co-vary naturally so that when females make their choices they will reinforce the correlation between the two, thus leading to phenotypic integration through correlational selection. Finally they show that plumage signals are honest in the sense that they are backed up by physiological readiness to elevate T and thus the likely intensity of an aggressive encounter.

4) **To assess sexual independence by comparing T’s role in the activation of aggressive behavior.** Sex differences in the hormonal mediation of the same trait predict sexual independence; similarities in mediation predict correlated responses and the potential for constraint.

Experiments that are partially completed are assessing whether repeated intrusions cause residents females to elevate T (Schrock et al. in prep., they do not), and whether other hormones, e.g., progesterone, are involved in the mediation of female aggression (Jawor et al. in prep.). Jawor et al. (in prep) compared hormones levels prior to and after an intrusion by a
female conspecific and a heterospecific, and preliminary data suggest that being presented with a same sex conspecific led to an increase in female progesterone but not testosterone (Jawor et al. 2006, Jawor et al. in prep.). The progesterone results appear below.
MAJOR FINDINGS OF THE PROPOSED RESEARCH (these are presented in relation to the objectives described in the proposal, using the same order as the activities file and the same headings)(published findings are referred to as citations, as yet unpublished findings are presented as graphs)

1) To experimentally elevate testosterone (T) in females, measure the phenotypic effects, and compare them to previous results from males.

Based on a study completed prior to the award (Clotfelter et al. 2004), we had concluded that experimental elevation of T in females did not reduce nest defense as it had in males (Cawthorn et al. 1998). However the data on females were obtained during incubation, and males do not incubate, they only feed nestlings. So a better comparison would be to compare females treated with testosterone (T-females) to control females (empty implants, C-females) during the nestling stage of reproduction. When presented with a stuffed nest predator (a chipmunk), females of both treatments dive at the mount, sometimes making contact (a hit), and also return periodically to look in the nest (a check), presumably to determine whether there are still young to defend. Results from O’Neal et al. 2008 showed that T-females with nestlings dived at the stuffed predator less frequently than C-females did but did not differ in hits or checks.

This conclusion was strengthened when a principal component analysis was used to consolidate the behavioral data and the two studies were compared directly. Control females increase the intensity of nest defense when caring for nestlings as opposed to eggs while testosterone treated females do not and the interaction is significant (O’Neal et al. 2008).

We pursued other studies assessing the impact of T on females and comparing them to males. Below is a tabular summary of phenotypic traits shown to be influenced by experimental elevation with testosterone and a comparison of the sexes for whether or not they were similarly sensitive to the elevation.

<table>
<thead>
<tr>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attractiveness ++</td>
<td>Attractiveness -</td>
</tr>
<tr>
<td>Song++</td>
<td>Incubate young</td>
</tr>
<tr>
<td>Feeding young --</td>
<td>Feeding young</td>
</tr>
<tr>
<td>Defend nestlings --</td>
<td>Defend nestlings --</td>
</tr>
<tr>
<td>Immune function --</td>
<td>Immune function --</td>
</tr>
<tr>
<td>Corticosterone, CBG ++</td>
<td>Corticosterone, CBG ++</td>
</tr>
<tr>
<td>Molt --</td>
<td>Molt --</td>
</tr>
</tbody>
</table>

++ = Enhanced by T  
- = suppressed by T  
No symbol = unaffected by T

Summarized in Ketterson et al. 2009
The conclusion is that the sexes resemble one another in their sensitivity to T in some traits and not in others; the implication is that they are ‘partially free’ to evolve independently, but not entirely.

2) To determine fitness consequences of experimentally elevated T in free-living females. Proposed studies will provide evidence of adaptive or maladaptive outcomes of elevated T in females.

The fitness aspect of these studies is partially published in O’Neal et al 2008 who report greater rates of nest predation of testosterone-treated females than controls. The other data will be summarized as part of Nicole Gerlach’s thesis, which will be completed this year.

The first figure below compares T- (red) and C- (yellow) females for proportion treated that were found to have built nests, which demonstrates reduced fecundity. The second figure below compares females by treatment for annual return, which suggests no effect on survival. These data and other data in the works on mating success appear to reveal fecundity costs in relation to experimentally elevated T in females, and these costs have the potential to act as a constraint on male evolution.

% females nesting (X² = 8.77, df = 1, p = 0.003)

% females returning (X² = 0.05, df = 1, p = 0.823)
3) **To assess individual variation in hormonal responsiveness and the potential for correlated response to selection.**

This aspect of our work has been particularly exciting, and we have made major progress publishing our results. We learned first that T in response to a standardized injection of gonadotropin releasing hormone (GnRH) is a repeatable property of individuals (Jawor et al. 2007, General and Comparative Endocrinology). Further, baseline T prior to GnRH injection co-varies with immune function (levels of IgG and complement, Greives et al. 2007, Functional Ecology). In addition, elevation of T in response to a GnRH challenge co-varies with a sexually selected plumage character (tail white, McGlothlin et al., 2007, Journal of Evolutionary Biology) and with the degree to which T increases naturally after a simulated territorial intrusion (McGlothlin et al., 2007, Journal of Evolutionary Biology).

One of our goals was to determine whether individual variation in T relates to the trade-off between mating and parental effort that we had seen using experimental manipulations. Our results show that males that elevate T more in response to GnRH are more aggressive towards intruders and less attentive to their offspring, just as we would expect if the trade-off were mediated naturally by testosterone (McGlothlin et al., 2007, American Naturalist).

Collectively, we believe these results are truly important. First, they validate earlier experimental results that revealed relationships among immune function, aggression, and T in male juncos. But they go farther in showing that natural endogenous levels of T co-vary positively with aggression and negatively with immune function and parental behavior. Thus they provide support for hormonal mediation of a trade-off between traits that are related to reproduction (mating success) and survival. The implication, which would require verification and also assumes no important variation in male condition, is that individuals living in situations that call for frequent elevations of T in response to social cues would have reduced ability to fight off infections.

These findings also complement earlier experimental results, which showed that females prefer males with higher T and whiter tails and that when these traits are presented in opposition, females are split in their preferences. The current data show that tail white and T co-vary naturally so that when females make their choices they will reinforce the correlation between the two, potentially leading to phenotypic integration through correlational selection. Finally they show that plumage signals are honest in the sense that they are backed up by physiological readiness to elevate T and thus the likely intensity of an aggressive encounter.

Results to date have not compared aggression and parental behavior in relation to response to GnRH in the same individuals, and we attempted to determine whether they apply at the level of the individual. We gathered preliminary data on response to GnRH, courtship and aggression. We did observe that individual males that sing more during a simulated territorial intrusion also sing more when presented with a captive female to court, but the experiment will need repeating because the endocrine data were not good (Schulz, McGlothlin et al. in prep.).

Finally with respect to males we learned that survival selection on response to GnRH by males is stabilizing, and the same is true for fecundity selection (McGlothlin et al. submitted).
to Am Nat, under review) (survival $\beta = 0.12$ $\gamma = -0.30*$; fecundity $\beta = 0.15$, $\gamma = -0.31*$)(see figure for survival selection). These data are unique and represent important findings.

Another area in which we have made major progress is in the relationship between female response to GnRH and maternal effects. We learned the females elevate T in response to GnRH only during the week before they lay eggs in a period we have termed the ‘golden week.’ At that time not only does T rise in response to GnRH, but the degree to which it rises co-varies with the concentration of T in the eggs the female lays (Jawor et al., 2007 *Functional Ecology*). Work on female response to GnRH is ongoing.

4) To assess sexual independence by comparing T’s role in the activation of aggressive behavior. Sex differences in the hormonal mediation of the same trait predict sexual independence; similarities in mediation predict correlated responses and the potential for constraint. To address these issues we compared results of experimental elevation of T to the study of social modulation of T.

Somewhat paradoxically, at least upon initial consideration, experimental elevation of T enhances aggression in female juncos (Zysling et al. 2006), but simulated territorial intrusions do not lead to an elevation in T (Jawor et al. 2006). These results appear at first to be contradictory, but in fact they may reflect the way in which links in the pathway between environment and phenotype can be altered by the evolutionary process, leaving portions of the pathway intact but untapped.
Further experiments conducted by Schrock and Jawor assessed whether repeated intrusions cause resident females to elevate T (Schrock et al. MA thesis; they do not), and whether other hormones, e.g., progesterone, are involved in the mediation of female aggression (Jawor et al. in prep; they are not).

Finally, graduate student, Kristal Cain picked up this aspect of the work, and she is collected field data to assess phenotypic correlates in females of response to GnRH. These include morphological variation (e.g. digit ratio, body size, and tail white) and behavioral variation (response to predator, response to female intruder). These studies are still underway.
NSF final report, research and education activities

History and goals

Since 1986 our research group has studied the integrating effect of the steroid hormone testosterone on behavior and physiology in a songbird. Our subject is the Dark-eyed Junco (Junco hyemalis), and we study free-living and captive individuals in Virginia, Indiana, and more recently, California and South Dakota. In the early years of the project our research focused on males, and that work continues, though in a new framework. The work covered by this award focused on primarily on testosterone in females.

The long-term goal of the research has been to take an experimental approach to the evolution of life histories by manipulating hormones and measuring the effects of the manipulation on phenotype and fitness in male and female juncos. In particular, we addressed the trade-off between mating and parental behavior and between reproduction and survival.

The approach is to experimentally elevate the hormone testosterone and then to compare the behavior, physiology, and performance of manipulated and control phenotypes. This comparison allows us to quantify how selection maintains adaptive distributions of phenotypes (e.g., if the manipulated phenotypes have lower fitness than controls) or whether organisms are sometimes hormonally constrained in their ability to respond to selection (if altered phenotypes have higher fitness than controls). The latter possibility might hold, at least in ecological time, because of the tendency of hormones to produce pleiotropic effects, some of which may be disadvantageous if hormones are altered. For example, if males would benefit from higher levels of testosterone, but the consequences for females would be disadvantageous, correlated responses to selection could constrain male evolution.

The research supported by this award addressed this possibility by comparing the sexes for their sensitivity to experimentally elevated testosterone. This award also allowed us to take another tack in our research on males, which was to assess variation among males in their tendency to elevate testosterone naturally and also to assess the relationships between naturally varying testosterone and phenotypic traits, concentrating on traits already known to be affected by experimental elevation of testosterone. Thus, research conducted under this award asked whether males with naturally greater ability to elevate testosterone after a challenge were, as would be predicted by experimental studies, less parental and more aggressive towards other males than were males with naturally lower value of testosterone.

Specific objectives

We cited four objectives in the proposal that led to this award.

1) To experimentally elevate testosterone (T) in females, measure its phenotypic effects, and compare them to previous results from males. New experiments will address T’s effect on female attractiveness and parental behavior towards nestlings. Studies of
hand-raised offspring will reveal whether experimentally elevated maternal T influences offspring plumage.

2) To determine fitness consequences of experimentally elevated T in free-living females. Proposed studies will provide evidence of adaptive or maladaptive outcomes of elevated T in females.

3) To assess individual variation in hormonal responsiveness and the potential for correlated response to selection. Planned studies will show whether response to GnRH co-varies with phenotype or fitness. Greater response in individuals with more mates or greater fecundity would suggest a role for T in sexual selection; study of relatives will indicate potential for correlated responses.

4) To assess sexual independence by comparing T’s role in the activation of aggressive behavior. Planned experiments will assess whether repeated intrusions cause resident females to elevate T in response to an intruder or whether other hormones mediate female aggression and competition.

Activities in relation to these specific objectives

1) To experimentally elevate testosterone (T) in females, measure its phenotypic effects, and compare them to previous results from males. We captured close to 100 females during each of the years 2005, 2006, and 2007 and treated them with subcutaneous implants of T or with empty implants to produce controls. We released them and observed their behavior at the nest (e.g., parental behavior, nest defense). During 2008 and 2009 we analyzed data, made presentations, and published results.

Under this objective, the award provided funds to support research training for two post-doctoral students (Jawor, Whittaker), five graduate students (Gerlach, O’Neal, Schrock, Reichard, Cain), six REU undergraduate students (Reichard, Young, Houdeck, Ainsworth, Schultz, Spevak) and three field assistants in the period between undergraduate and graduate school (Knox, Bessler, Reichard).

2) To determine fitness consequences of experimentally elevated T in free-living females. For the females implanted with T or with empty implants, we worked in the field, and we measured fecundity (nest predation, likelihood of producing a clutch, clutch size, reproductive success), mating success (number of females to mate/not mate, number of sires of offspring as measured by microsatellite analysis), and survival (presence/absence in a late summer census during the summer of implant, likelihood of being present during censuses conducted the next breeding season). Some of these results have been published (O’Neal et al. 2008) and some are still in preparation when the award expired. With respect to training, Gerlach and O’Neal were the primary people on this goal.

Research training opportunities were funded as described under objective 1.

3) To assess individual variation in hormonal responsiveness and the potential for correlated response to selection. The objective here is to quantify variation among individuals in their capacity to elevate testosterone when challenged with a releasing
hormone, gonadotropin releasing hormone (GnRH). GnRH ordinarily stimulates the release of luteinizing hormone (LH), which leads to the release of testosterone (T). We captured individuals in the field in Virginia, bled them to determine pre-challenge levels of T, injected (challenge) them with GnRH, collected blood samples 30 min post-challenge, and measured T. We then related the rise in T to attributes of the birds, including parental behavior, aggressiveness, plumage coloration and natural levels of antibodies.

In 2007 and 2008, we extended the work to two populations of juncos residing in southern California, one on the campus of the University of California, San Diego, and one in the nearby Laguna Mountains. In 2007-2009 we also initiated study of a population of juncos that resides in the Black Hills of South Dakota and that work is ongoing.

Research training under this objective included one post-doctoral student (Whittaker), three graduate students (Atwell, Bergeon, Cain), four REU students (Campbell-Nelson, Spevak, Ainsworth, Shapiro) and a number of field assistants in Virginia, South Dakota, and California.

4) To assess sexual independence by comparing T’s role in the activation of aggressive behavior. We also conducted resident-intruder trials with captive females to determine whether T rises in response to an intrusion, and if it does not, what other hormones might. Females were bled prior to their introduction into a breeding aviary, allowed to mate and then bled again after an intruder was introduced. Intruders were either female conspecifics or heterospecifics that served as controls. We compared changes in plasma levels of T and other hormones (estradiol, progesterone), prior to and after the intrusions.

This project provided research training for former post-doctoral student Jawor, now an Assistant Professor at Southern Mississippi University and undergraduates she helped to train (Young)

Additional research and educational activities not mentioned specifically in the award proposal

1) Seasonal variation in volatile compounds found in preen oil. Using HPLC we compared preen oil constituents in juncos by sex, season and population. The data were published in Chemical Ecology and have served as the basis for future work on major histocompatibility complex (MHC) variation in isolated populations.

Research training was received by graduate student Schrock for the initial study, and during year 3 and during the extension, post-doctoral student Whittaker and graduate student Atwell began a comparison of preen oil composition in the two California populations and they are relating that variation to patterns of inbreeding, extra-pair fertilizations, and MHC.
2) *Response of HPG to kisspeptin, is this peptide important in regulating avian reproduction?* We asked whether injected kisspeptin led to increased levels of LH and testosterone as we would expect if this peptide plays a role in the regulation of seasonal reproduction. Graduate student Schrock received training.