Final Report for Period: 03/1998 - 02/2003
Submitted on: 08/21/2003
Principal Investigator: Ketterson, Ellen D.
Award ID: 9728384
Organization: Indiana University
Title:
Using Hormones to Explore Adaptation and Constraint in a Male Bird

Project Participants

Senior Personnel

Name: Ketterson, Ellen
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Nolan, Val
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Parker, Patricia
Worked for more than 160 Hours: Yes
Contribution to Project:
Project has subcontract to Dr. Parker, she and her student, Nidia Arguedas, are measuring genetic reproductive success of T- and C-male juncos.

Name: McNabb, Anne
Worked for more than 160 Hours: No
Contribution to Project:
Dr. McNabb, Department of Biology, Virginia Tech University, has conducted radioimmunoassays of thyroid hormones in junco plasma. To date we have provided the plasma and she has performed the assays without requesting financial support from this award.

Name: Eklund, Amy
Worked for more than 160 Hours: Yes
Contribution to Project:
Research Associate salary paid by Center for the Integrative Study of Animal Behavior, development of microsatellites for paternity analyses

Post-doc

Name: Buerkle, Alex
Worked for more than 160 Hours: Yes
Contribution to Project:
collaborating on a sex ratio project, performed PCR reactions, ran gels and statistical analyses, helped to write manuscript

Name: Neudorf, Diane
Worked for more than 160 Hours: Yes
Contribution to Project:
Neudorf was in charge of a sub-project designed to compare the behavior of females mated to T-and C-males at the times they are fertile. She oversaw the work of D. Ziolkowski.

Name: Clotfelter, Ethan
Worked for more than 160 Hours: Yes
Contribution to Project:
Conducts experiments on whether treatment with T affects food consumption and food choice by males when feeding themselves and when feeding offspring. His salary is paid by the NSF-supported Center for the Integrative STudy of Animal Behavior. His

Name: Sandell, Maria

Worked for more than 160 Hours: Yes

Contribution to Project:

Maria visited from Lund University, Sweden and from Cornell. She is studying yolk steroids in red-winged blackbirds and budgerigars with Anne Clark and Elizabeth Adkins-Regan and she joined our lab to learn about yolk steroids in relation to female aggression.

Name: Reed, Wendy

Worked for more than 160 Hours: Yes

Contribution to Project:

Conducted research on analyzing long-term demographic data and headed up a project on egg composition

Name: Duffy, Deborah

Worked for more than 160 Hours: Yes

Contribution to Project:

Studied effect of social status on mate choice by female juncos

Graduate Student

Name: Jones, Kerry

Worked for more than 160 Hours: Yes

Contribution to Project:

in charge of data collection on sub-project

Name: Grindstaff, Jennifer

Worked for more than 160 Hours: Yes

Contribution to Project:

NSF pre-doctoral fellowship, researched impact of T in male juncos on immune function in females

Name: Arguedas, Nidia

Worked for more than 160 Hours: Yes

Contribution to Project:

perform genetic analyses of relatedness, student Ohio State University

Name: Lipar, Joe

Worked for more than 160 Hours: Yes

Contribution to Project:

participates in assays of yolk steroids, helped to capture subjects for a captive colony of juncos

Name: Monk, Daniela

Worked for more than 160 Hours: Yes

Contribution to Project:

One of the people measuring genetic reproductive success of juncos, will spend spring semester at Ohio State University with Nidia Arguedas in Patty Parker's lab

Name: Smulders, Tom

Worked for more than 160 Hours: Yes

Contribution to Project:

Smulders was a graduate student working with Tim DeVoogd at Cornell University. He did a collaborative project with us during the summer of 1997, and during the award period he analyzed data and presented a poster at the Society for Neuroscience. He received no salary from the award.

Name: Heidinger, Britt

Worked for more than 160 Hours: Yes

Contribution to Project:

Supported by Departmental funds and Center for the Integrative Study of Animal Behavior, researched response of captive breeding females to intrusion by another female

Undergraduate Student

Name: Wolf, Wendy

Worked for more than 160 Hours: Yes

Contribution to Project:

undergraduate researcher, tail white and age

Name: Sanders, Johnnie

Worked for more than 160 Hours: Yes

Contribution to Project:

Conducted research on co-variation between endogenous levels of testosteorne and behavior/physiology of male and female dark-eyed juncos

Name: Gaudioso, Jackie

Worked for more than 160 Hours: Yes

Contribution to Project:

Field assistant at MLBS, researched impact of T in females on incubation behavior

Name: Hill, Katie

Worked for more than 160 Hours: Yes

Contribution to Project:

Field assistant at MLBS, learned to use GPS to locate nests and netting locations

Name: Grieves, Tim

Worked for more than 160 Hours: Yes

Contribution to Project:

supported by departmental funds, researched impact of sex ratio on immune function in captive breeding juncos

Technician, Programmer

Other Participant

Name: Casto, Joseph

Worked for more than 160 Hours: Yes

Contribution to Project:

Casto heads up the sub-project on immune responses of T- and C-male dark-eyed juncos and also heads up sub-project on yolk steroids. He acts as both a researcher and as support personnel. He is completing a Ph.D at Johns Hopkins University

Name: Snajdr, Eric

Worked for more than 160 Hours: Yes

Contribution to Project:

project manager in the field, data support in the off season

Name: Aron, Carol

Worked for more than 160 Hours: Yes

Contribution to Project:

Name: Kleffner, Jennifer

Worked for more than 160 Hours: Yes

Contribution to Project:

Name: Hill, Michelle
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Ziolkowski, David
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Munn, Kelly
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Donnelly, Elise
Worked for more than 160 Hours: No
Contribution to Project:

Name: Garrison, William
Worked for more than 160 Hours: Yes
Contribution to Project:
Field assistant at Mountain Lake Biological Station, summer 1999.

Name: Farhat, Tabussum
Worked for more than 160 Hours: Yes
Contribution to Project:
Ms. Farhat helped to care for juncos at Kent Farm Bird Observatory and helped to quantify their behavior during breeding, measuring eggs, weighing offspring, etc.

Research Experience for Undergraduates

Name: Parker-Renga, Ian
Worked for more than 160 Hours: Yes
Contribution to Project:
Ian conucted experiment asking whether experimental elevation of testosterone affects the attractiveness of females to males in dark-eyed juncos. Support came from the award. In 2000-2001 he was both an REU student and supported by this award

Name: Hopenstand, Dafna
Worked for more than 160 Hours: Yes
Contribution to Project:
Undergraduate researcher, effects of testosterone on neural regions assoiated with vision

Name: Hagen, Erin
Worked for more than 160 Hours: Yes
Contribution to Project:
Erin was an NSF-supported REU student at Mountain Lake Biological Station. She worked with Neudorf on female behavior during the period when they are fertile.

Name: Schubert, Kristin
Worked for more than 160 Hours: Yes
Contribution to Project:
conducted research on effect of mouth color on parental behavior, Mountain Lake Biological Station, summer 2000

Years of schooling completed: Junior
Home Institution: Other than Research Site

Home Institution if Other: William and Mary

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

Fiscal year(s) REU Participant supported: 2000

REU Funding: REU site award

Name: Joel, McGlothlin

Worked for more than 160 Hours: Yes

Contribution to Project:

conducted research on effect of testosterone on female mate choice, Mountain Lake Biological Station, summer 2000

Years of schooling completed: Junior

Home Institution: Other than Research Site

Home Institution if Other: Vanderbilt University

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

Fiscal year(s) REU Participant supported: 2000

REU Funding: REU site award

Name: O'Neal, Dawn

Worked for more than 160 Hours: Yes

Contribution to Project:

An REU in 2000 (site award to Mtn Lake Biological Station, field assistant in 2001 on this award, researched impact of T in females on nest defense

Years of schooling completed: Junior

Home Institution: Other than Research Site

Home Institution if Other: Washington University

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

Fiscal year(s) REU Participant supported: 2001

REU Funding: REU site award

Name: Elitzur, Ben

Worked for more than 160 Hours: Yes

Contribution to Project:

An REU in 2000 (site award to Mtn Lake Biological Station), researched impact of T on female incubation

Years of schooling completed: Freshman

Home Institution: Other than Research Site

Home Institution if Other: University of California, Berkeley

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

Fiscal year(s) REU Participant supported: 2001

REU Funding: REU site award

Name: Wolf, Wendy

Worked for more than 160 Hours: Yes

Contribution to Project:

An REU (supplement to this award) in 2000, field assistant in 2001, researched impact of plumage manipulation on male mate choice in juncos

Years of schooling completed: Junior

Home Institution: Same as Research Site

Home Institution if Other:

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

Fiscal year(s) REU Participant supported: 2001

REU Funding: REU supplement

Name: Faurot, Michael

Worked for more than 160 Hours: Yes

Contribution to Project:

REU supported by site grant to Center for the Integrative Study of Animal Behavior, investigated impact of T in eggs on metabolic rate and locomotion in japanese quail, advised by Wendy Reed

Years of schooling completed: Junior

Home Institution: Other than Research Site

Home Institution if Other: Washburn University

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

Fiscal year(s) REU Participant supported: 2002

REU Funding: REU site award

Organizational Partners

University of Virginia Main Campus

Project conducted at Mountain Lake Biological Station which is administered by the University of Virginia

Ohio State University Research Foundation

Indiana U has subcontract with Ohio State U

Lund University

Faculty member, Dennis Hasselquist, is collaborating with us on a project inolving assaying of immune function in birds

Virginia State University

Dr. McNabb performs assays to detect thyroid hormone in junco plasma.

University of Missouri-Saint Louis

Other Collaborators or Contacts

Dennis Hasselquist, immunological assays, University of Lund, Sweden
Victor Apanius, immunological advice, Florida International University

Activities and Findings

Research and Education Activities:

Major research and education activities of the project.
IBN-9728384 to Ellen D Ketterson and Val Nolan Jr., Indiana University, Bloomington, $375,277, 03/01/98 û 02/28/02, ôUsing Hormones to Explore Adaptation and Constraint in a Male Bird.ö
Research supported by this award addressed effects of experimentally elevated testosterone on the behavior, physiology, and fitness of males of a socially monogamous songbird, the dark-eyed junco (Junco hyemalis: Emberizidae). We used hormone implants to raise plasma levels of testosterone to the maximum values that occur naturally at the time breeding begins and held them there, creating a seasonal pattern that typifies males of polygynous species (Wingfield et al., 1990). In controls, plasma levels of testosterone peaked in early spring, then declined to lower levels, as is true of juncos naturally. Prolonged experimental elevation of testosterone produced phenotypes (or phenocopies) that are rare in natural populations but that lie within the ôgenomic capacityö of the organism because they can be induced by the treatment. We characterized the experimental phenotype (T-males) in relation to controls (C-males) and asked why the experimental phenotype is

not more common in nature. We had previously shown that testosterone mediates a trade-off between mating effort and parental effort: T-males court more, are more attractive to females, and provide less care for offspring. They are also more successful at siring young through extra-pair fertilizations, but less successful at rearing young with their social mates. During the period covered by this award we found that testosterone suppresses immune function and reduces survivorship. We also identified an array of traits that are and are not sensitive to hormonal manipulations. We believe the work has contributed significantly to understanding of the evolution of complex adaptations, particularly those mediated by hormones and involving trade-offs. Its impact can be assessed in terms of contribution to human resources, enhancement of education/outreach/potential society implications, and contributions to the scientific literature, as described in the other sections of this report.

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

Training and Development:
YEAR 1

One research associate (Casto also a graduate student at Johns Hopkins University), one post-doctoral student (Neudorf), six graduate students (Jones, Lipar, Monk, Aron, Grindstaff, and Arguedas, Ohio State University) and three field assistants (Ziolkowski, Hill, Kleffner), many of whom typically go on to graduate school, received training during the first year of this award.

New techniques and who learned them during the first year:

1. We imported techniques for measuring cell-mediated and humoral immunity from Johns Hopkins University (with help from Sabra Klein and Randy Nelson). We can now use these techniques in the lab for this and other projects. Three participants in the project who became fully proficient or who learned a portion of the technques are J. Casto, J. Grindstaff, and J. Kleffner.

2. A former student, Alex Buerkle, now a post-doctoral student at Indiana University (under Loren Rieseberg) taught J. Casto and J. Grindstaff how to run PCR reactions and gels in order to determine the sex of nestling birds from blood samples. The technique relies on a probe for a sex-specific marker (one that binds to avian sex chromosomes, in particular to introns that differ in length on the homomorphic and heteromorphic sex chromosomes). Our lab has used molecular techniques in the past to measure relatedness (DNA fingerprinting), but this is our first use of PCR and our first experience with molecular sexing.

Already established techniques and who learned them during the first year of the award:

1. Measuring yolk steroids - research associate, Joe Casto.

2. Field methods for studying behavior and demography of dark-eyed juncos - graduate students and field assistants, C. Aron, J. Grindstaff, M. Hill, J. Kleffner, D. Ziolkowski.

3. Recording nestling vocalizations in the field and video taping behavior - graduate student and field assistant, C. Aron, M. Hill.

4. Measuring oxygen consumption, food consumption, and body temperatures, scoring molt, K. Jones.

5. Recording behavior of birds carrying radio transmitters in the field - postdoctoral student D. Neudorf who was already experienced with these techniques which she applied to a new species and taught to D. Ziolkowski.

Training and Development

YEAR 2

One new faculty member at Sam Houston University and former post-doc (Neudorf), one research scientist at Indiana University and former post-doc (Schoech), research associate (Casto also a graduate student at Johns Hopkins University), two post-doctoral students (Clotfelter, Sandell), four graduate students (Jones, Lipar, Grindstaff, and Arguedas, Ohio State University), three field assistants (Snajdr, Ziolkowski, now a graduate student with Robert Ricklefs at U Missouri-St. Louis, and Bill Garrison), two NSF REU students, Hopenstand and Smith and 2 undergraduates, W. Wolf and I Parker-Renga received training during year 2 of the project.

New techniques and who learned them during the second year:

1. Schoech purchased the Linco multi-species leptin assay and used it with mixed success on plasma from 4 bird species. Casto participated.

2. Clotfelter used video cameras and adobe photoshop to quantify mouth coloration in nestling birds and neck collars to collect food from nestlings and he and taught the techniques to other members of the crew.

3. Casto used NIH image to measure plumage coloration and taught the technique to undergraduate W. Wolf; he also used to to measure the volume of brain nuclei from prepared sections and taught the technique to REU undergraduate D. Hopenstand

Already established techniques and who learned them during the second year of the award:

1. Measuring yolk steroids - visiting post-doc from Sweden, Maria Sandell.

2. Field methods for studying behavior and demography of dark-eyed juncos - graduate students and field assistants, W. Garrison, D. Hopenstand, E. Smith.

3. Lab methods for elevating plasma T, observing behavior and analyzing video tapes, statistical analysis, taught by Ketterson and Jones to undergraduate I. Parker-Renga.

YEAR 3

1. Project members served as mentors to 3 REU students during summer of 2000, Joel McGlotlin, Ian Parker-Renga, and Kristin Schubert. All three will be authors on papers resulting from their work. Collectively they learned experimental design, methods for presenting scientific findings, videotaping and analysis, immune assays, behavioral observation, and field methods.

Outreach Activities:

YEAR 1

1. Mountain Lake Biological Station holds an open house, and in July, 1998, the field crew prepared a display of our methods for the public and led bird walks for the visitors.

2. Ketterson invited by T. Donovan, SUNY Syracuse, to speak to her Women in Science Class regarding challenges and rewards of a career in science.

3. Ketterson served as reviewer for NRC report on Hormonally Active Agents in the Environment.

YEAR 2

1. Presentation of research to Kinsey Institute, Indiana University.

2. Visited laboratory of Indian scientist when attending meeting in Varanasi, India. Ketterson will try to help him make connections in the US.

3. Taught seminar in endocrine disrupting chemicals in the environment to help build ties between basic and applied environmental science at Indiana University.

YEAR 3

Major contribution is effort devoted to Center for the Integrative Study of Animal Behavior and promotion of the study of animal behavior.

Journal Publications

Lipar, J.L., Ketterson, E.D., V. Nolan Jr., and J.M. Casto., "Egg yolk layers vary in the concentration of steroid hormones in two avian species.", General and Comparative Endocrinology, p. 220, vol. 115, (1999). Published

Ketterson, E.D., and V. Nolan Jr., "Adaptation, exaptation, and constraint: a hormonal perspective.", American Naturalist, p. S4, vol. 153, (1999). Published

Hill, J.A., Enstrom, D.E., Ketterson, E.D., and V. Nolan Jr., "Mate choice by captive female dark-eyed juncos: static vs. dynamic traits.", Behavioral Ecology, p. 91, vol. 10, (1999). Published

Schwagmeyer, P.L. and E. D. Ketterson, "Breeding synchrony and EPF rates: the key to a can of worms?", Trends in Ecology and Evolution, p. 47, vol. 14, (1999). Published

Lipar, J., Ketterson, E.D., and V. Nolan Jr., "Intra-clutch variation in testosterone content of eggs of red-winged blackbirds.", Auk, p. 231, vol. 116, (1999). Published

Schoech, S., Ketterson, E.D., and V. Nolan Jr., "Exogenous testosterone and the adrenocortical response in the Dark-eyed Junco, Junco hyemalis.", Auk, p. 64, vol. 116, (1999). Published

Smulders, T.V., Casto, J.M., Ketterson, E.D., Nolan, V. Jr. and DeVoogd, T.J., "Testosterone and captivity affect telencephalic and hippocampal volume in dark-eyed juncos (Junco hyemalis). (abstract)", Society for Neuroscience Abstracts, p. 683, vol. 24, (1998). Published

Schoech, S., Buntin, J., Sharp, P. Ketterson, E.D., and V. Nolan Jr., "The effect of exogenous testosterone on parental behavior, plasma prolactin, and prolactin binding sites in dark-eyed juncos.", Hormones and Behavior, p. 1, vol. 34, (1998). Published

Cawthorn, J.M., Morris, D., Ketterson, E.D., and Nolan, V. Jr., "Influence of elevated testosterone on nest defence in dark-eyed juncos.", Animal Behaviour, p. 617, vol. 56, (1998). Published

Kast, T.L., Ketterson, E.D., and V. Nolan Jr.
, "Variation in ejaculate quality in dark-eyed juncos according to season, stage of reproduction, and testosterone treatment.", Auk, p. 684, vol. 115, (1998). Published

Lipar, J., and E.D. Ketterson, "Interlayer variation in steroid concentration within the yolks of dark-eyed junco (Junco hyemalis) eggs. (abstract)", American Zoologist, p. 21A, vol. 38, (1998). Published

Lynn, S.E., Houtman, A.M. Weathers, W.W., Ketterson, E.D., and V. Nolan Jr., "Testosterone increases activity but not daily energy expenditure in captive male dark-eyed juncos (Junco hyemalis).", Animal Behaviour, p. 581, vol. 60, (2000). Published

Smulders, T.V., Casto, J.M., Nolan, V. Jr., Ketterson, E.D., and T.J. DeVoogd, "Effects of testosterone and captivity on the volumes of four brain regions in the dark-eyed junco (Junco hyemalis)", J. Neurobiology., p. 244, vol. 43, (2000). Published

Hudman, S.P., Ketterson, E.D., and V. Nolan Jr., "The effects of experimentally elevated testosterone on prevalence of coccidia in male dark-eyed juncos.", The Auk, p. 1048, vol. 117, (2000). Published

Lipar, J.L., and E.D. Ketterson, "Yolk testosterone concentration is correlated with the mass of the musculus complexus in red-winged blackbird nestlings.", Proceedings of the Royal Society of London B., p. 2005, vol. 267, (2000). Published

Casto, J.M., Nolan, V. Jr., and E. D. Ketterson., "Steroid hormones and immune function: experimental studies in wild and captive dark-eyed juncos (Junco hyemalis).", American Naturalist, p. 408, vol. 157, (2001). Published

Clotfelter, E.D., V. Nolan Jr., and E.D. Ketterson, "Effects of elevated testosterone and food deprivation on food consumption and prey size preferences in male dark-eyed juncos (Junco hyemalis).", Ethology, p. 439, vol. 107, (2001). Accepted

Grindstaff, J.L., Buerkle, C.M., Casto, J.M., Nolan, V. Jr., and E.D. Ketterson., "Male attractiveness and offspring sex ratio in dark-eyed juncos (Junco
hyemalis).", Behavioral Ecology and Sociobiology, p. 312, vol. 50, (2001). Published

Clotfelter, E.D. Schubert, K.A. Nolan, Val. Jr., and E.D. Ketterson., "The signal function of mouth colour in nestling dark-eyed juncos (Junco hyemalis).", Animal Behaviour, p. , vol. , ( ). Submitted

Neudorf, D.L. Ziolkowski, D J. Jr. Nolan, V. Jr., Ketterson, E.D., "Movement by female dark-eyed juncos during the fertile period suggests that

males visit females for extra-pair copulations.", Ethology, p. 1, vol. 108, (1). Published

Clotfelter, E.D. Schubert, K.A. Nolan, V. Jr., and E.D. Ketterson, "Mouth colour signals thermal stress in nestling dark-eyed juncos (Junco hyemalis).", Ethology, p. 171, vol. 109, (2003). Published

Wolf, W., Casto, J.M., Nolan, V. Jr., and E. D. Ketterson., " The effect of female ornamentation on mate choice by male dark-eyed juncos", Animal Behaviour, p. , vol. , (2004). Accepted

Books or Other One-time Publications

Ketterson, E.D., Nolan, V. Jr., Casto, J.M., Buerkle, C.A.,Clotfelter1,
E., Grindstaff, J.L., Jones, K.J., Lipar, J.L., McNabb, F.M.A.,
Neudorf, D.L., Parker-Renga, I., Schoech, S.J. and E. Snajdr., "Testosterone, phenotype, and fitness: a research program in evolutionary behavioral endocrinology", (2001). Book, Published
Editor(s): A. Dawson and C.M. Chaturvedi (eds.),
Collection: Avian Endocrinology.
Bibliography: Narosa Publishing House, New Delhi, India, in press.

Web/Internet Site

URL(s):

http://www.indiana.edu/~animal/research/ketterson.html
http://www.bio.indiana.edu/GradPrograms/EEB/facultyresearch.html;
http://www.bio.indiana.edu/Ketterson/kettersonlab.html;

Description:

The first site, developed by Martha Holder, who is in the employ of the NSF-supported Center for the Integrative Study of Animal Behavior, is an outstanding example of an academic website.

The second and third web pages were developed by the Department of Biology at Indiana University and briefly describe our research and that of people associated with the lab, some of whose work is supported by this award.

Other Specific Products

Contributions

Contributions within Discipline:

Tbe project lies at the intersection of evolutionary biology, comparative physiology, behavioral endocrinology, and behavioral ecology. The goal is to further understanding of how and why suites of hormonally mediated traits evolve and the approaches are both proxmate and ultimate. In 199, we wrote a discussion paper entitled Adaptation, Exaptation and Constraint: a hormonal perspective, that appeared in the American Naturalist. In it synthesizeed the utility we see in including physiology, particularly endocrinology, in the evolutionary synthesis. A supplementary paper expanding on this theme will appear in 2001:

Ketterson, E.D., Nolan, V. Jr., Casto, J.M., Buerkle, C.A.,Clotfelter1, E., Grindstaff, J.L., Jones, K.J., Lipar, J.L., McNabb, F.M.A., Neudorf, D.L., Parker-Renga, I., Schoech, S.J. and E. Snajdr. 2001. Testosterone, phenotype, and fitness: a research program in evolutionary behavioral endocrinology. Pp. 19-40, in A. Dawson and C.M. Chaturvedi (eds.), Avian Endocrinology. Narosa Publishing House, New Delhi, India/

Contributions to Other Disciplines:

Please see preceding section.

Contributions to Human Resource Development:

Contribution to the development of human resources.ùNumbers of students receiving training with the help of IBN-9728384, 1998-2001: 9 post-doctoral associates (4 male, 5 female), 8 graduate students (2 male, 6 female, 1 Hispanic), 21 undergraduate or post-undergraduate students (9 male, 12 female, 1 African-American, 3 first-generation; 11 supported by REU programs, 9 already gone on to graduate school, and 8 are co-authors on submitted manuscripts or publications).

Post-doctoral associates: A Buerkle (now Asst Prof., U of Wisconsin, Eau Claire), E Clotfelter (now Asst. Prof., Amherst College), D Monk (now at Washington State U), D Neudorf (now Asst. Prof., Sam Houston State U), M Sandell (now Researcher, Lund U, Sweden), S Schoech (now Asst. Prof., Memphis U), W Reed (now Asst. Prof., North Dakota State U), J Casto (now Director of Research, Midwest Neurological Foundation), D. Duffy (SIT)

Graduate students: N Arguedas (Ohio State U, now post-doc, Ohio State), K Jones (employed in industry), J Lipar (now post-doc, Washington State U), T Smulders (Cornell U, now faculty, University of Newcastle upon Tyne), S Raouf (now visiting faculty, Oklahoma State University), B VanRoo (now Asst. Prof. Framingham State University), J Grindstaff (SIT), B Heidinger (all SIT)

Undergraduate research students and field assistants: C Aron (completed MS, Indiana U, now US govÆt employee), E Donnelly (William and Mary, now grad student, UC, Santa Cruz), B Elitzur (U Cal, Berkeley), M Faurot (Washburn University), W Garrison, T Grieves (Indiana U), J Hill (since Ph.D., U of New Mexico), K Hill (Colorado State University), D Hopenstand (UC, Berkeley), E Hagen (Truman State U), M Hill (since grad student, U of Vermont), S Hudman (since grad student, U of Vermont), J Kleffner, S Lynn (since Ph.D., U of Washington, now Visiting Asst Prof. Colby College), J McGlothlin (now grad student, Indiana U), D OÆNeill (Washington U, St. Louis), I Parker-Renga (Indiana U, now school teachr), J Sanders (Indiana U), K Schubert (William and Mary, now grad student, Queens U), D Ziolkowski (now grad student, U of Missouri, St. Louis), W Wolf (Indiana U, now Medical School Indiana U).

Contributions to Resources for Research and Education:

The fact that NSF funds our research enhances our ability to gain institutional support from Indiana University for research facilites.

During 1999, a program known as the Research Facilities Fund (RFF) supported an upgrade to the Ketterson-Nolan and Rod Suthers animal facilities at Indiana University's Kent Farm for the second year in a row. The upgrade, relied first on an RFF award of $37,840 and supplements from the Department of Biology ($7,000) and the Program in Medical Sciences ($7,000) and led to the construction of new outdoor cages for studying bird behavior and a generator to insure continual electrical power. A supplement of $12,000 in 2000 led to the completion of the outdoor cages and enhanced ability to control lighting indoors (we now have separate day length controls for 11 holding areas, whereas before we did not have independent control of each holding room). The net effect is that we have outstanding facilities for studying birds at Indiana University.

Contributions Beyond Science and Engineering:

Categories for which nothing is reported:

Any Product

Contributions: To Any Beyond Science and Engineering

MAJOR RESEARCH ACTIVITIES (citation to work supported by this NSF award in bold, see section of project report entitled Publications and Products).

OVERVIEW

Since 1987, we have studied the effects of testosterone (T) on the behavior, physiology, and fitness of males of a socially monogamous songbird, the dark-eyed junco (*Junco hyemalis:* Emberizidae).

Fieldwork is conducted at Mountain Lake Biological Station in Virginia. Each spring before breeding begins we randomly assign half the males we capture to receive subcutaneous silastic implants packed with testosterone (experimental or T-males). The other half receives empty implants (control or C-males). The implants raise plasma levels of testosterone in the experimental animals to the maximum values that occur naturally at the time breeding begins and hold them at the maximum for several months. In controls, plasma testosterone peaks at the same time as in the experimentals, i.e., in early spring, but then declines to lower levels as is true of juncos naturally. Prolonged experimental elevation of T produces phenotypes (or *phenocopies*) that are rare in natural populations but that obviously lie within the "genomic capacity" of the organism, because they arise in response to the treatment.

Our goal has been to characterize the experimental phenotype and to ask why it is not more common in nature. More generally the goal has been to enhance understanding of natural selection and the evolution of complex adaptations, particularly those mediated by hormones.

We anticipated four classes of outcomes:

(1) Naturally existing phenotypes (controls, C-males) would outperform experimentals (T-males) with respect to most measures of survival and reproduction, which would lead us to conclude that the existing array of phenotypes is maintained by natural selection and that naturally arising deviant phenotypes would rapidly be selected against.
(2) Control phenotypes would outperform experimentals on some measures of fitness, but not on others. If controls performed better overall (total fitness), the persistence of the existing array of phenotypes would be explained, but it would also suggest that this array is "suboptimal" or constrained because alternative combinations of traits might perform better.
(3) Experimental phenotypes would outperform controls in total fitness. In this case, the rarity of naturally occurring phenotypes in nature that would be counterparts to our experimental animals would present a paradox and provide a different kind of evidence of constraints on phenotypic evolution.
(4) Experimental and control males would not differ detectably in fitness, either on a component-by-component basis or overall, which would indicate either that selection is intermittent and/or that a broad range of phenotypes is capable of using alternative means to achieve similar success.

In a series of papers based on results obtained prior to the current award and summarized in Ketterson et al. 1996, **Ketterson and Nolan 1999, and Ketterson et al. 2001**, we demonstrated that experimentally elevated testosterone causes males to sing more, to be more attractive to females and to increase home range size. T also depresses male parental behavior, which, in turn, leads females to compensate for the shortfall by increasing their own parental effort (Ketterson et al. 1992, Chandler et al. 1994, 1997, Enstrom et al. 1997). Physiologically, testosterone enhances levels of corticosterone and corticosterone-binding globulin, depresses spring body mass and fat, suppresses pre-basic (autumn) molt, and has no effect on sperm numbers (Ketterson et al. 1991, Nolan et al. 1992, Klukowski et al. 1997, Kast et al.

1998). As for effects of these changes on male fitness, T-males obtain *more* extra-pair fertilizations (EPFs) with females mated to other males than C-males, but achieve *lower* reproductive success with their social mates because nestlings die during development (Raouf et al. 1997). In sum, treatment with testosterone alters components of male fitness by affecting the balance of mating effort and parental effort, but these effects have been counteracting, and there has been no net reduction or enhancement of reproductive fitness.

In other words, the results were closest to outcome 2), above, because controls outperformed experimentals on some measures and experimentals outperformed controls in others. But the results did not conform exactly with any of the predictions because neither the T- nor the C-phenotype outperformed the other in total fitness. Testosterone had clear and predictable phenotypic consequences but the connection to fitness was fuzzy. In that way, the findings were also partially consistent with outcome 4), which predicted that alternative phenotypes could achieve similar reproductive success. They were also consistent with intermittent selection.

New directions

As we assessed where we were, we realized that the situation was far more complex than we had initially envisioned. First, the closer we looked, the more phenotypic effects of T we found. We became intrigued: how many fitness-related traits were responsive to T and how did they relate to one another? Apparently if we were to predict how selection might act on the experimental phenotype, we would need a thorough catalog of the hormone's consequences. We did not see this as a strictly descriptive exercise, but rather an effort to investigate the meaning of the term "trait" in the context of hormones.

We also realized that it would be necessary to understand how – in the mechanistic sense – testosterone was inducing its effects. Understanding the consequences of selection on single hormonally mediated traits could not be done without considering how that selection would affect the mechanisms underlying trait expression. Just as quantitative genetics provides a framework for studying the effect of selection of correlated traits, we felt the need for a similar framework for studying how physiology affects the response to selection.

For example, testosterone predictably causes males to become more active. It also increases home range size in the field and makes captive males more attractive to females. Are these observations all inter-related? That is, does hyperactivity translate into home range size and attractiveness? If yes, a complete understanding of the situation would require knowledge of how T enhance locomotor activity. What is the pathway – is it direct or indirect, etc.

Another indication that interpreting the outcome of selection on our experimental phenotype was more complex than we had anticipated was the observation that the effects of the treatment resonated through the population. T affected males by altering their physiology, *but it also affected the physiology and behavior of untreated conspecifics*. The male's phenotype extended beyond his own body and was reflected in that of his mates and offspring. This unanticipated set of considerations deserved more attention.

We also needed a way to test whether selection on T-mediated traits was consistent or intermittent. As a first step, we took a correlative approach by investigating annual variation between fitness and treatment in already collected data.

These issues are summarized in the research objectives outlined below. They have structured the research of the past three years.

Major research activities of the past three years have focused on the following 5 objectives

Objective #1 - to further characterize the effects of experimental elevation of T on the male phenotype (e.g., effects of T on immune function, timing of molt, components of parental behavior);

Objective #2 - to investigate *how* T has its effects on the phenotype (e.g., by influencing the secretion of other hormones or density of hormonal receptors, or by effects on neuroanatomy);

Objective #3 - to determine how T's effects on male behavior and physiology influence the phenotype of females and offspring. We referred to such effects as the male's **extended phenotype**. For example, would females alter the sex ratio of their offspring when mated to attractive T-males, would they vary egg components when mated to T-males which are poor parents, etc.

Objective #4 - to relate altered T to fitness by means of long-term comparisons of the relative survival and reproductive success of experimental and control males;

Objective #5 - to identify the most productive directions for future research,

We completed some additional projects with NSF support and have reported our results in various scientific forums, and we report on these activities at the end of this section.

Objective # 1 – to further characterize the effects of experimental elevation of T on the male phenotype

Immune function.—Based on empirical data from other species and on theoretical predictions (e.g. Folstad and Karter 1992), research associate Joe Casto anticipated that treatment with T might suppress immune function in T-males and, ultimately, susceptibility to disease and survival. Consequently, we exposed T- and C- males to an immune challenge using three techniques:

1) a test of cell-mediated immunity: injection of a plant lectin phytohemaglutinin (PHA) into the wing (procedures modified from Lochmiller et al. 1993) to produce swelling; a study of captive and free-living males (**Casto et al. 2001**).
2) a test of humoral immunity (IgM): injection with sheep red blood cells (SRBC) and subsequent measurement of antibody production with a hemagglutination assay (procedures of Hay and Hudson, 1989; Ros et al., 1997); a study of captive males (**Casto et al. 2001**).
3) another test of humoral immunity (IgG): injection with keyhole limpet hemocyanin (KLH) followed by an ELISA to assess antibody production; a study of captive males (**Casto et al. in prep**.).

Incidence of disease.— Similarly and in collaboration with a field assistant, Steve Hudman, we asked whether T- and C-males differed in the incidence of infection with *Isospora*, a protozoan parasite that lives in the gut and induces a wasting disease known as coccidiosis. The parasite's presence can be detected because it sheds reproductive oocysts in avian feces. Early and late in two breeding seasons we collected feces from T- and C-males and examined them for the presence/absence of oocysts (**Hudman et al. 2000**).

Activity, food consumption, and metabolic rate.— Data from mammalian and other bird species suggest that testosterone increases activity, food consumption and metabolic rate. All these effects might mediate male trade-offs between self-maintenance and reproductive effort and in collaboration with Anne Houtman (supported by an NSF ROA award), Wes Weathers, and REU student Sharon

Lynn, we established two groups of males and compared their behavior by quantifying activity levels and feeding and by using doubly labeled water to compare daily energy expenditure (**Lynn et al. 2000**)

Components of male parental behavior.— T's suppressive effect on male parental behavior (incubation or feeding of nestlings) is well established in a variety of species, but little is known about exactly which components of parental behavior are affected. E.g. does T reduce a male's motivation to forage in general or his tendency to deliver food to young? Does he respond differently to food items that are appropriate for self-feeding or for feeding of young? In collaboration with post-doctoral associate Ethan Clotfelter, we offered mildly food-deprived males a choice of whether to spend time eating or courting females. We also offered them a choice of food items that differed in size (**Clotfelter et al., in press**).

In a related project currently being conducted in the field, Clotfelter is asking whether T affects the size or number of prey items delivered by males to nestlings (research underway).

Molt and other traits expressed during the non-breeding season—Evolution of potentially beneficial T-mediated traits might be constrained if breeding and non-breeding levels of T were correlated and individuals exhibiting high T-mediated trait values while breeding were more likely to exhibit those traits during the non-breeding season when they might be detrimental. Consequently, graduate student Kerry Jones quantified some of the effects of T during autumn and winter. We had four treatment groups of captives. We used a smaller dose of T than during breeding, and we implanted the first two groups of males, one T- and one C-, at the beginning of autumn when post-breeding molt normally begins and the other two groups after molt was complete. We compared the timing and extent of molt as well as food consumption (**Jones et al., completed Master's thesis and manuscript**).

Objective #2 - to investigate *how* T has its effects on the phenotype

Interactions with other hormones.—Understanding the evolution of hormone-mediated traits requires consideration of how one hormone affects the action of others. If selection were, for example, to favor individuals with higher levels of trait mediated by one hormone, how might that affect the secretion/half-life, response to other hormones and thus the traits they mediate? In collaboration with Anne McNabb of Virginia Tech University, graduate student Kerry Jones compared T- and C-males for plasma concentrations of the thyroid hormone (T_3 and T_4) prior to and during the autumn molt (**Jones et al., completed Master's thesis and manuscrip**t). In collaboration with former post-doctoral associate, Steve Schoech, and with John Buntin (University of Wisconsin-Milwaukee) and Peter Sharp (Rosalin Institute, Scotland), we compared plasma concentrations of corticosterone, prolactin, and leuteinizing hormone in free-living breeding T- and C-males (**Schoech et al. 1998, 1999, Ketterson et al. 2001**).

Neuroanatomy.— Greater home range size in males treated with testosterone (Chandler et al. 1994) led us to ask whether T would affect the volume of the hippocampus, a region of the brain closely associated with spatial memory. In collaboration with Tom Smulders, then a graduate student at Cornell, we conducted a combination field/lab study in which implanted males of both treatments were allowed to live free or held captive. After a period of 6-8 weeks, we collected the males and later sectioned their brains to look for neuroanatomical consequences (**Smulders et al. 2000**).

Objective #3 - to determine how T's effects on male behavior and physiology influence the phenotype of females and offspring - male's extended phenotype.

Egg size, egg steroids.—Defining the boundary of the phenotype is a controversial topic (Dawkins 1982), and we asked how T-treatment of the male influences the phenotype of the females he associates with and the offspring he produces. Working in the field, we compared egg size of females mated to T- and C-males (**t- and c-females**). Because another study reported that female zebra finches (*Poephila guttata*) mated to attractive males deposit more androgens in their eggs (Gil et al.1999), we also compared concentrations of egg steroids (**Casto et al. in prep**.). In particular, recalling that T-male juncos are more attractive (Enstrom et al. 1997, Hill et al. 1999), we asked whether yolk androgens were more concentrated in the eggs of t-females (**Casto et al. in prep**.).

Female home range size.— Given the greater incidence of extra-pair fertilizations in females mated to controls, and in collaboration with then post-doctoral associate, Diane Neudorf, we asked whether females mated to C-males were more likely to leave their home territories to mate with neighboring T-males or whether T-males were more likely to intrude on the territories of C-males to copulate with their mates. To pursue this question we radiotracked 14 females and measured and compared the home range size of t- and c-females during nest-building (**Neudorf et al. manuscript completed**).

Progeny sex ratio.—To determine whether differences in attractiveness of males would affect the sex ratio of young produced by their female mates (as found in other species by Burley 1986, Ellegren et al. 1996, Sheldon et al. 1999), we compared brood sex ratios of females mated to T- and C-males during the breeding seasons of 1995-1998. We extracted DNA from 204 blood samples from 51 full broods (broods with no egg or nestling mortality), used PCR to amplify an intron in the CHD gene that differs in length in the heteromorphic sex (females), thus allowing us to use gel electrophoresis to determine nestling sex at hatching (two bands = females, one band = male) (**Grindstaff et al. in press**).

Female stress response and immune function.— We knew already that females mated to T-males (t-females) compensated for decreased male parental care by increasing their own rate of feeding (Ketterson et al. 1992). We investigated whether this increase was stressful by asking whether the time-dependent rise in CORT that occurs in response to handling would be steeper in t-females than in c-females (**Schoech et al. 1999**). Having found a difference – the rise in t-females was steeper - and knowing than enhanced CORT in T-males suppressed immune function (**Casto et al. 2001**), during the summer of 2000, we undertook a study of immune responses of females mated to T- and C-males, using the assays previously employed with males (**Casto et al. in prep.).**

Mouth coloration of nestlings.— To investigate how reduced male parental care by T-males influences signaling of hunger by young and what stimuli might cause females to compensate for decreased male parental care, post-doctoral collaborator Ethan Clotfelter compared begging behavior and mouth coloration of nestlings of t- and c-females (**Clotfelter et al. in prep**.).

Objective #4 - to complete analysis of our long-term data on the relative survival and reproductive success of experimental and control males;

Reproductive success.—In collaboration with Patricia G. Parker of Ohio State University, her graduate student N. Arguedas, and postdoctoral associate D. Monk, we used mini- and microsatellites to measure annual variation in genetic reproductive success of T- and C-males. Lab work is complete on blood samples from 446 young belonging to 162 families from three breeding seasons (1994-96), and data analysis has begun.

Life span.—With respect to life span, based on a sample of 449 juncos implanted over 5 years, annual rate of return to the study area (assumed to equal survival) of T-males was 14% vs. 31% for C-males ($p<0.001$). While this clearly suggests a cost to T, interpretation is complicated. When the sample is confined to those males known to be present in late summer (n=266), there is no detectable effect of T on rate of return (55% for T-males, 62% for C-males, n.s.). Analysis is still underway.

Objective #5 - To identify new directions for this research

(1) Future research on this system will be more feasible if we can breed juncos in captivity. We are interested in individual variation and in resemblance among relatives in hormones and hormone-mediated traits – questions that will be more effectively pursued on captives. Since 1998 we have worked to improve our methods for captive breeding.

(2) Some indicators of individual phenotypic quality are dynamic, in that they vary over time as condition varies. Other indicators of quality are static, in that they persist even if quality declines. Behavioral traits tend to fall in the first category, while morphological traits such as appearance (e.g., plumage) tend to fall in the second. Steroid hormones can influence both kinds of traits, but at least among birds, steroids are better known as determinants of behavior. In some instances they have been shown to affect plumage coloration, but not in others. We have described sex and age-related variation in a plumage trait in juncos that plays a significant role in courtship displays (' tail white') **Casto, Wolf et al. in prep**.), and we have assessed its effect on female mate choice (**Hill et al. 1998**) and whether it or dynamic behavioral traits play a greater role in mating preferences. In 2000 we conducted a study of the effect of enhanced tail while on male mate choice (**Wolf et al., completed manuscript**). We are working towards a generalized understanding of how hormones affect and reflect various aspects of phenotypic quality.

(3) To date our research has focused on the effects of T on males. Our interests are now focused on females and we have begun descriptive and experimental studies on the effect of T on female behavior and physiology (**Parker-Renga et al. completed manuscript, Neudorf et al. completed manuscript).**

Additional projects completed

(1) After finding that yolk steroids vary with laying order in the red-winged blackbird (*Agelaius phoniceus*), graduate student Joe Lipar undertook an experimental study of T's effect on the 'hatching' or 'begging' muscle of nestlings (*musculus complexus*) and showed that experimental enhancement of yolk T caused the muscle's mass to increase, while experimental application of the T-antagonist, flutamide, reduced muscle mass (**Lipar et al. 1998 2000**).

(2) Interest in yolk steroids in birds has grown rapidly, but surprisingly few descriptive data are available about variation in concentrations across species, within species, and even within the yolk itself. Lipar section yolk in two species, juncos and red-winged blackbirds, and found that progesterone concentration varies across the layers of an egg's yolk, e.g., most concentrated in the yolk laid down earliest by the female and less concentrated in later-produced yolk (Lipar et al.1999). He also found variation according to layer in steroids, T and estradiol, but the pattern was different (**Lipar et al. 1999**).

Other research-related activities – invited talks, contributed talks, invited seminars, symposia organized

Invited meeting talks

1998 Invited presentations (2): recipient of Margaret Morse Nice medal for Excellence in Ornithological Research (with V. Nolan Jr.), joint meeting Ornithological Societies of North America; Keynote speaker, Midwest Animal Behavior Society meeting, Bowling Green, OH

2000 Invited symposium presentation (with V. Nolan Jr. and several co-authors), International Symposium on Avian Endocrinology, 2/2000, Varanasi, India

2000 Invited Baerands Lecturer, Dutch Behavioral Biologists Conference, Netherlands

Oral or poster presentations at meetings of scientific societies

1998 Contributed papers/posters presented (4) at American Ornithologists' Union (Hudman, Schoech presenting), Society for Integrative and Comparative Biology (SICB)(Casto presenting), Society for Neuroscience (Smulders presenting)

1999 Contributed papers/posters at professional meetings (5), American Ornithologists' Union (Neudorf, Ziolokowski presenting) Society for Integrative and Comparative Biology (SICB) (Lipar, Casto presenting) and Society for Behavioral and Neural Endocrinology (SBN) (Casto presenting)

2000 Contributed papers at professional meeting (3), Society for Integrative and Comparative (SICS)(Casto presenting), Society for Behavioral and Neural Endocrinology (SBN)(Casto presenting) Society for the Study of Evolution (Ketterson presenting).

Invited seminars

1998 (1) University of Oklahoma

1999 (3) SUNY-ESF, University of Wisconsin, University of Washington

2000 (3)Purdue University, Johns Hopkins University (Psychology Department), Kinsey Institute, Indiana University

Invited seminar: Michigan State University

Organized symposium

2000 Organized Symposium and round table for International Ethological Conference on Non-Genetical Inheritance, Tuebingen, Germany

MAJOR RESERCH FINDINGS (citation to work supported by this NSF award in bold; see section of project report entitled Publications and Products) for complete citations).

Major findings of the past three years as they relate to the objectives laid our in section of this report entitled Research Activities)

Objective #1 - to further characterize the effects of experimental elevation of T on the male phenotype (e.g., effects of T on immune function, timing of molt, components of parental behavior);
Objective #2 - to investigate *how* T has its effects on the phenotype (e.g., by influencing the secretion of other hormones or density of hormonal receptors, or by effects on neuroanatomy);
Objective #3 - to determine how T's effects on male behavior and physiology influence the phenotype of females and offspring. We referred to such effects as the male's **extended phenotype**. For example, would females alter the sex ratio of their offspring when mated to attractive T-males, would they vary egg components when mated to T-males which are poor parents, etc.).
Objective #4 - to relate altered T to fitness by means of long-term comparisons of the relative survival and reproductive success of experimental and control males;
Objective #5 - to identify the most productive directions for future research,

Objective # 1 – to further characterize the effects of experimental elevation of T on the male phenotype

Immune function.—

(1) In the immune project headed by J. Casto, we found that captive breeding male juncos treated with testosterone (T-males) were not as capable as controls (C-males) of mounting a cell-mediated immune response. In subjects released into the wild and later recaptured, we found less wing-web swelling in T-males than in C-males (70% less, $p = 0.034$, Casto et al. 2001). Among captives, however, T and C-males did not differ in wing-web swelling (**Casto et al. 2001**)
(2) Captive T-males were less effective than C-males at mounting a humoral response to SRBC (36% lower titers, one-tailed $p < 0.05$) (**Casto et al. 2001**). Treatment groups did not differ in antibody production after injection with KLH (**Casto et al. in prep**.).
(3) Efforts to manipulate corticosterone directly to see whether it or T is responsible for immune suppression were not fully successful in 1999, and we are deciding how best to pursue the question of which hormone is more important in suppressing immunity (**Casto et al. in prep**.).

Incidence of disease.— While the incidence of coccidial infections differed significantly over two years, using our methodology we found no difference in incidence between T- and C-males and therefore no connection between T's ability to suppress cell-mediated immune function (Casto et al. 2001) and the likelihood that males treated with testosterone would be infected with coccidia (**Hudman et al., 2000**).

Activity, food consumption, and metabolic rate.— We found that activity levels were greater in T- than in C-males (**Lynn et al. 2000),** as was food consumption (**Lynn et al. 2000, see also Clotfelter et al. 2001, Jones, KJ, Master's thesis, cited in Ketterson et al. 2001**, but paradoxically, metabolic rate as measured by doubly labeled water did not differ (**Lynn et al., 2000**). Our results are similar to those found in white-crowned sparrows and in another study of juncos and suggest that birds may compensate for enhanced daytime energy costs by decreasing nocturnal costs (**Lynn et al. 2000**). The question deserves further study.

Components of male parental behavior.— We found no detectable effect of T on whether mildly food-deprived males elected to eat or court females. Interestingly, while eating, T-males consumed more food than C-males and also chose larger food items **(Clotfelter et al., 2001**), which might predict differences in prey choice in the field (**Clotfelter et al. 2001).**

Molt and other traits expressed during the non-breeding season.— We found that testosterone delayed or completely suppressed molt in T-males (**Jones, KJ, completed Master's thesis, cited in Ketterson et al. 2000)**, compare Nolan et al. 1992). It also increased food consumption.

Objective #2 - to investigate about *how* T has its effects on the phenotype

Interactions with other hormones: effects of experimentally elevated testosterone on plasma levels of other hormones.—

(1) Testosterone had no detectable effect on T_3 or T_4, which suggests that T does not prevent or delay molt by suppressing secretion of thyroid hormones (**Jones, KJ, completed Master's thesis, cited in Ketterson et al. 2000)**.
(2) Levels of corticosterone (CORT)(sometimes referred to as the "stress hormone) were consistently higher in T- than C-males, as was the initial glucocorticoid response to the stress of handling (**Schoech et al. 1999**). This strongly suggests that some of the observed phenotypic effects of T are mediated by CORT (**Casto et al. 2001**).
(3) T- and C-males did not differ in concentration of prolactin (PRL, the "parental hormone") or in the extent to which prolactin receptor sites were occupied by PRL in the pre-optic area of the brain (**Schoech et al. 1998**). These similarities in PRL indicate that T's suppression of parental behavior should not be attributed to an effect of T on PRL secretion or activity. T interferes with parental behavior in some other way.
(4) T- and C-males did not differ in plasma concentration of leuteinizing hormone (LH) (T-males=0.97 ng/ml, C-males=1.32 ng/ml, n= 10, 12, p=0.256)(**Ketterson et al. 2001**). The similarity in LH indicates that our implants do *not* induce super-normal levels of T that suppress gonadotropin secretion.

Neuroanatomy.— In **Smulders et al. (2000),** we reported that captive juncos had a smaller hippocampal formation (HF) (both in absolute volume and relative to telencephalon) than free-living birds, regardless of hormone treatment. Testosterone-treated males (both captive and free-living) had a smaller telencephalon and nucleus rotundus, but not a smaller HF or ectostriatum, than controls. In agreement with earlier experiments we found that free-living testosterone-treated males had larger home ranges than free-living controls, but we found no corresponding difference in HF volume.

Objective #3 - to determine how T's effects on male behavior and physiology influence the phenotype of females and offspring.

Egg size, egg steroids.— Egg size of females mated to T- and C-males (*t- and c-females*) differed: eggs of t-females are smaller than eggs of c-females (egg width differed by ca ~8%, $p < 0.04$) (**Casto et al. 1999, in prep**.) Interestingly, this effect on egg size was stronger early in the season when weather and food supply are less favorable (Casto et al., 1999, MS in prep). Despite the difference in egg size, which might predict differences in female allocation to eggs, there was no effect of male treatment on the concentrations of three yolk steroid, estradiol, testosterone, or progesterone (**Casto et al. 1999, in prep**.).

Female home range size.—To determine whether male treatment affects female tendency to seek extra-pair matings, D. Neudorf and her assistant D. Ziolkowski radiotracked females and measured and compared the home range size of t- and c-females during nest-building. We have completed home-range measurements of ~14 females and analysis of the GPS data are underway (**Neudorf et al., 2002**).

Progeny sex ratio.—Differences in attractiveness of males induced by hormonal manipulations did not affect the sex ratio of young produced by their female mates. The proportion of females among T-broods was 0.507; among C- broods it was 0.505 (**Grindstaff et al., in press.).**

Female stress response and immune function.— In keeping with our prediction based on steeper stress responses in females mated to T-males than in females mated to C-males we found suppressed cell-mediated immune function in the t-females (**Casto et al. in preparation**). This suggests a cost to females that mate with more attractive males that provide less parental care. To the extent that males are mate-limited, this cost to females may serve to constrain male evolution.

Mouth coloration of nestlings.— Clotfelter and REU student K. Schubert compared mouth coloration of nestlings of t- and c-females to investigate how reduced male parental care by T-males influences signaling of hunger by young. Mouth color did not differ according to treatment of the male, but it did increase with time out of the nest, regardless of treatment of how much nestlings were fed. Mouth color may be a signal of thermal stress (**Clotfelter et al. 2003**).

Objective #4 - to complete analysis of our long-term data on the relative survival and reproductive success of experimental and control males;

Reproductive success.— Recently obtained data on extra-pair fertilizations, when combined with data collected 1990-1993 (Raouf et al. 1997), show that percentage of young produced by EPFs varies annually from 13-43% and that a reliable predictor of this variation is annual variation in the rate of nest predation ($n=7$ years, $p < 0.04$). Further, T- and C-males are affected differentially, with C-males apparently more affected by the presence of T-males during years of low predation. T- and C-males are equally likely to lose paternity to EPFs, but the addition of more data confirmed the finding that T-males are more likely to sire young in the nests of other individuals. Several possible explanations for this pattern will be considered in a manuscript under preparation (Reed et al. in prep.).

Life span.— Results of mark-recapture analysis indicate that T-males have lower annual survival than C-males. Results from Program MARK reveal that the most parsimonious statistical model (based on AIC) includes different survival rates for T-male and C-male groups (0.38 ± 0.03 versus 0.44 ± 0.03), but no differences in capture probability. Survival and capture probability did not vary with year, and age effects were not detected on either survival or capture probability. All models tested had adequate goodness of fit (probability of observed deviance < 0.7) based on bootstrap tests performed with 200 replicates (Reed et al. in prep).

The balance of reproduction favors C-males except when extra-pair copulations are taken into account; in that case T-males have greater fitness (lambda) than C-males, leaving open the (oversimplified) question of why male juncos do not evolve towards higher levels of testosterone.

Objective #5 - To identify new directions for this research,

(1) Many questions of interest are difficult to answer in the field and we have been making intense efforts to breed juncos in captivity that are finally paying off handsomly. In our first effort during

the summer of 1999 we established 11 breeding pairs at the Kent Farm Bird Observatory, an indoor aviary. All the females paired and built nests, and most laid eggs though often not in nests or in identifiable clutches. Incubation was unpredictable and only 4 pairs (36%) hatched young and 0 fledged young. We learned about the nutritional and other nesting requirements of the females and were more successful during 2000 when 4 of 8 pairs hatched young (50%) and 2 of 8 fledged young (25%). Still, females were prone to pitch young from the nest at hatching. Between 2001-2003 we improved the food supply and nesting sites and have achieved predictable success. More than half of the pairs we form now undertake reproduction and almost all those that lay eggs follow though by incubating and feeding hatchings. After 4 years of experimenting we are now able to plan experiments that involve captive breeding in juncos, e.g., how does sex ratio affect readiness to breed, do dominant females prevent subordinates from breeding? We are most interested in pursuing issues related to maternal effects, i.e., how does the maternal environment affect development of offspring, e.g., via yolk steroids?

(2) Some indicators of individual phenotypic quality are dynamic, in that they vary over time as condition varies. Other indicators of quality are static, in that they persist even if quality increases of declines. Behavioral traits tend to fall in the first category, while morphological traits such as appearance (e.g., plumage, antlers) tend to fall in the second. Steroid hormones can influence both kinds of traits, but, at least among birds, steroids are better known as determinants of behavior than plumage. We have described sex and age-related variation in a plumage trait in juncos that plays a significant role in courtship display (' tail white') (**Casto, Wolf et al. in prep**.), and we have assessed its effect on female mate choice (**Hill et al. 1998)**. In 2000 we asked whether experimentally enhanced tail white would affect male mate choice and found that it did not (**Wolf et al. in press**). We are working towards a generalized understanding of how hormones affect and reflect various aspects of phenotypic quality, both dynamic and static. A current project involves measuring the relative strength of natural and sexual selection on tail white **(McGlothlin et al. in prep).**

(3) To date much of our research has focused on the effects of T on males. Our interests are now focused on females, and we have begun descriptive and experimental studies of the effect of T on female behavior and physiology (**Parker-Renga et al. completed manuscript, McGlothlin et al. completed manuscript, Clotfelter et al. completed manuscript)**. For example, prior studies in juncos have shown that T-males are more attractive to females than C-males, which might predict that selection would favor testosterone-mediated traits in males. In 1999, however, we found that experimentally elevating testosterone in females (n=8) made them *less* attractive to males (n=30, $p < 0.05$), probably because it induced male-typical behavior in females (song)(**Parker-Renga et al. completed manuscript, briefly described in Ketterson et al. 2001).** In 2000 we found that females treated with T were less discriminating in mate choice trials than control females (**McGlothlin et al. 2002**). In 2002-20002 we found that testosterone does not affect incubation or nest defense in female juncos (**Clotfetler et al. completed manuscript**). We are studying these phenomena in the context of correlated responses to selection in one sex acting as an impediment to evolution in the opposite sex.

Additional projects completed

Yolk steroids in red-winged blackbirds

After finding that yolk steroids vary with laying order in the red-winged blackbird (*Agelaius phoniceus*), graduate student Joe Lipar then studied T's effect on *musculus complexus* (the 'hatching' or 'begging' muscle of nestlings) and showed that experimental enhancement of yolk T caused the muscle's mass to increase, while experimental application of the T-antagonist, flutamide, reduced muscle mass (**Lipar et**

al. 1998, 2000). (2) In juncos and red-winged blackbirds, we found that steroid concentration varies across the layers of an egg's yolk (**Lipar et al.1999**).

Hormones and parental behavior in blue-headed and red-eyed vireos

In a comparison of two related species living sypatrically, testosterone declines earlier in the season/nestling cycle in the species in which males incubate (blue-headed vireos) and later in the species in which males do not incubate (red-eyed vireos)(**Van Roo et al. in press).**