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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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# #507 Experimentally Elevated Testosterone in Male Dark-eyed Juncos Suppresses Cell-mediated Immune Function of Social Mates and Offspring

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## 1. Recent evidence from birds suggests that parental immune function covaries with parental effort.

Increased parental effort reduces immunocompetence of adults birds.

- Adult Great Tits (*Parus major*; Allander 1997)
  - brood size manipulated experimentally
  - prevalence and intensity of *Haemoproteus* infection increased with brood size
- Adult Zebra Finches (*Taeniopygia guttata*; Deerenberg et al. 1997)
  - brood size manipulated experimentally
  - antibody response to immunization with sheep erythrocytes decreased as brood size increased
- Female Collared Flycatchers (*Ficedula albicollis*; Nordling et al. 1998)
  - brood size manipulated experimentally
  - antibody response to immunization with Newcastle Disease virus decreased as brood size increased
  - intensity of *Haemoproteus* infection increased with brood size
- Female Pied Flycatchers (*Ficedula hypoleuca*; Moreno et al. 1999)
  - brood size manipulated experimentally
  - females fed more and weighed less as brood size increased
  - T lymphocyte cell-mediated immune response (wing-web swelling) decreased as brood size increased.

## 2. Evidence also suggests that nestling immune function covaries with parental effort.

Reduced per capita parental effort suppresses immunocompetence of nestlings:

- Nestling Barn swallows (*Hirundo rustica*; Saino et al. 1999)
  - brood size manipulated experimentally
  - T lymphocyte cell-mediated immune response (wing-web swelling) decreased as brood size increased.

## 3. When testosterone levels of male Dark-eyed Juncos are experimentally maintained at springtime maxima throughout the breeding season, manipulated males exhibit suppressed immune function, elevated corticosterone levels and reduced parental effort.

In comparison to control males, males with experimentally elevated testosterone levels:

- provision nestlings less frequently (Ketterson et al. 1992; Schoech et al. 1998).
- fledge fewer young from the nests of their social mates (Raouf et al. 1997).
- have higher baseline corticosterone levels, more robust adrenocortical responses to handling stress, and higher levels of corticosteroid-binding globulin (Ketterson et al. 1991; Klukowski et al. 1997; Schoech et al. 1999).
- have suppressed immune function despite their reduced parental effort (Casto et al. 2001).
- have social mates that compensate for their reduced parental effort (Ketterson et al. 1996; but see Schoech et al. 1998).



## 4. We assessed whether cell-mediated immune function of social mates and offspring of testosterone-treated males was suppressed in comparison to social mates and offspring of control males.

### Study population:

These studies were conducted on a population of juncos found near Mountain Lake Biological Station on Salt Pond Mountain in Giles County, VA (37° 22' N, 80° 32' W). The study population has been a part of a long term study on the effects of T in male juncos. The study of immune function in adult females was conducted during the 2000 breeding season. The study of nestling immune function was conducted during both the 1999 and 2000 breeding seasons.

### Early season implanting, and immune priming:

Beginning in mid-April of each breeding season we captured all adult juncos on our study site. Immediately following capture, we weighed all birds ( $\pm 0.1$  g) and then scored fat stores (0-5; Helms and Drury 1960) and pectoral musculature (1-3; Gosler 1991). Males were randomly assigned to a treatment group, anesthetized with methoxyflurane, and implanted subcutaneously with two Silastic® implants (1.47 mm i.d., 1.95 mm o.d.) filled with either 10 mm of crystalline T (T-males) or left empty (C-males or controls). Finally, we primed both males and females with a subcutaneous injection of 0.25 mg phytohemagglutinin (PHA) in 50  $\mu$ l of Freund's complete adjuvant (FCA; Sigma F-5881) into the right scapular apertium and an intraperitoneal injection of 2% sheep red blood cells (SRBC; PHA procedures modified from Smits et al. 1999; Casto et al. 2001; SRBC procedures modified from Hay and Hudson 1989; Casto et al. 2001).

### Immune challenges:

Throughout the summer we identified mated pairs, located their nests, recorded hatch dates, and measured nestling development. For nests found after hatching, nestlings were compared to population growth curves to estimate their ages (Wolf et al. 1988). PHA and SRBC challenge injections were administered on nest-day (ND) 6, PHA responses were measured on ND 7, and SRBC responses on ND 12, if nests remained active.

On the morning of ND 6, using a mist net, we captured the female at the nest. Immediately after capture we collected 200  $\mu$ l of blood and assessed hematocrit levels following centrifugation. After bleeding we weighed the birds and scored both pectoral musculature and fat stores. We then initiated two tests of acquired immune function. To test cell-mediated immune function, we took an initial measurement of the left wing-web thickness to the nearest 0.01 mm using a pressure sensitive digital thickness gauge (Mitutoyo model 547-500), and then made a subcutaneous injection of 0.25 mg of PHA in 50  $\mu$ l in PBS into the wing-web. To assess antibody production we administered an intraperitoneal injection of 100  $\mu$ l of 2% SRBC in PBS.

On ND 7, approximately 24 hours after the PHA challenge, we recaptured the female in the mist net using tape playback, or a stuffed nest predator. We remeasured the thickness of the left wing-web and then released the bird. The degree of wing-web swelling (cell-mediated immune response) was determined by subtracting the pre-challenge wing-web measurement from the post-challenge measurement. Because nest depredation was common and very few nests remained active through ND 12, we did not collect enough data to assess the impact of male treatment on female antibody responses to the SRBC challenge.

On the afternoon of ND 6 we measured tarsus length, wing chord, and mass of each nestling in a nest. We also banded each nestling for individual identification and measured thickness of its left wing web. Each nestling was then given a subcutaneous PHA challenge injection (as described above). On ND 7, approximately 24 hours after the PHA challenge, we revisited the nest, again recorded somatic measures, and remeasured wing-web thickness. The cell-mediated immune response was calculated as described above.



## 5. In social mates of testosterone-treated males, immune responses were significantly suppressed compared to social mates of control males.

- Females mated to T-males had significantly (41%) less wing-web swelling in response to a PHA challenge than did females mated to C-males (Mann-Whitney U = 3.0, n = 2, 8; p = 0.036; fig 1).
- Females mated to T-males had significantly lower pectoral condition scores on Nest Day 6 than females mated to C-males (Mann-Whitney U = 14.0, n = 2, 8; p = 0.013; fig 2).
- Females mated to T-males did not have significantly different body masses on Nest Day 6 than females mated to C-males (p = 0.79). All females tested had undetectable fat stores while caring for nestlings.

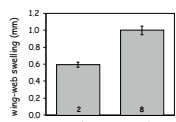


Fig 1. Differential wing-web swelling of parental female juncos mated to either T- or C-males

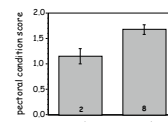


Fig 2. Pectoral condition scores of parental female juncos mated to either T- or C-males

## 6. In nestlings of testosterone-treated males, immune responses were significantly suppressed compared to nestlings of control males.



- Nestlings of T-males had significantly (19%) less wing-web swelling in response to a PHA challenge than did nestlings of C-males (t-test, df = 1,20; p = 0.025; fig 3).
- Nestlings of T-males tended to have greater body mass, tarsal lengths, and wing chords on Nest Day 6 and 7 than did nestlings of C-males; however in each instance the between treatment differences were small (<5%) and were not significantly different (Mixed ANOVAs, df = 1, 19; p's = 0.658, 0.059, and 0.260 respectively).

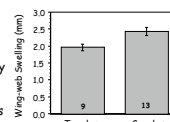


Fig 3. Differential wing-web swelling of nestling juncos of either T- or C-males

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## 7. Discussion of Results and Their Implications:

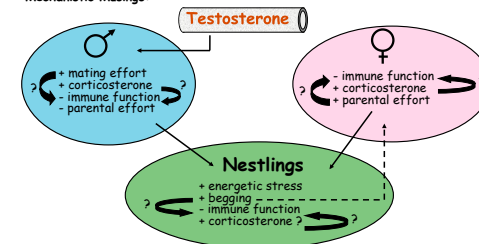
How might immunosuppression in social mates of T-males be mediated?

1. Female juncos increase their parental effort to compensate for reduced male provisioning of young (Ketterson et al. 1996; but see Schoech 1998), and increased parental effort has been linked with immune suppression. Energetic investment may be diverted away from immune function and toward parental effort in social mates of T-males.
2. Females mated to T-males have higher basal corticosterone titers than females mated to C-males (Schoech et al. 1999).
- Chronically elevated corticosterone has been shown to be immunosuppressive in both birds and mammals (Fowles et al. 1993; Dhabhar & McEwen 1999).
- Elevated corticosterone results in decreased pectoral muscle mass in juncos (Goy et al. 1990). This may explain why social mates of T-males also had lower pectoral muscle condition scores than the mates of C-males.

How might immunosuppression in nestlings of T-males be mediated?

1. Nestlings of T-males beg more often, more loudly, and for longer durations than nestlings of C-males (Kennedy et al. in prep).
- It is unclear whether begging is energetically expensive for passerine nestlings (McCarty 1996; 1997). If it is costly, increased begging may divert energetic resources away from immune function in T-nestlings.
- Begging can signal energetic stress. Experimentally-induced energetic stress leads to elevated basal corticosterone levels in nestling birds (Klitschky et al. 1999), which may itself be immunosuppressive.
2. In our study population significantly fewer nestlings fledged from nests of T-males than from nests of C-males (Ketterson et al. 1996).
- The lower fledging rate could be due to reduced immune function and potential associated increases in disease susceptibility of nestlings from T nests.

### Mechanistic Musings:



We thank the following for support of this research:

