Transcriptomic support for the Immunocompetence Handicap Hypothesis but not the Oxidation Handicap Hypothesis

Daniel J. Newhouse*, Ben J. Vernasco
1Department of Biology, East Carolina University, Greenville, NC, USA
2Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA
*Corresponding author: newhoused12@students.ecu.edu

Abstract

Sexually selected traits are hypothesized to be honest signals of individual quality due to the costs associated with their development or expression. Testosterone, a sex steroid known to influence the production of sexually selected traits, has been proposed to underlie the costs associated with sexually selected traits via its immunosuppressive effects (i.e., the Immunocompetence Handicap Hypothesis) or by influencing an individual’s exposure/susceptibility to oxidative stress (i.e., the Oxidation Handicap Hypothesis). Previous work testing these hypotheses has primarily focused on physiological measurements of immunity or oxidative stress, but little is known about the molecular pathways by which testosterone could influence immunity and/or oxidative stress pathways. To measure the molecular consequences of experimentally elevated testosterone, we used previously published RNA-seq data from studies that measured the transcriptome of individuals treated with either a testosterone-filled or an empty (i.e., control) implant. Two studies encompassing two species of bird and three tissue types fit our selection criteria. We found strong support for the Immunocompetence Handicap Hypothesis, but no support for the Oxidation Handicap Hypothesis. More specifically, testosterone-treated individuals exhibited strong signatures of immunosuppression, encompassing both cell-mediated and humoral immunity. Our results suggest that testosterone enforces the honesty of sexually-selected traits by influencing an individual’s immunocompetence rather than their exposure or susceptibility to oxidative stress.

Keywords
Androgens, testosterone, sexual selection, immunity, oxidative stress, transcriptomics

Introduction

There is a long-standing interest in understanding why sexually selected traits have evolved and one hypothesis suggests that mates have selected for traits that are costly to develop or bear (i.e., the handicap hypothesis; Zahavi, 1975). An important assumption of the handicap hypothesis is that an individual’s investment in sexually selected traits correlates with their investment in other traits that also influence their reproductive success or survival (Grafen, 1990; Andersson, 1994). Individuals face tradeoffs when fitness-related traits exhibit negative correlations and, as a result, individuals can incur survival costs from their reproductive investments (Stearns, 1992). These costs arise because the development and/or expression of traits important for reproduction (e.g., sexually selected traits) and traits important for survival (e.g., immune function) are dependent
on the same mechanism (Zera and Harshman, 2001). As such, our understanding of the evolution of sexually selected traits is dependent upon our understanding of the pleiotropic nature of the mechanisms that underlie their production (Kokko et al., 2003).

Testosterone is a sex steroid that is known to influence the development and/or expression of sexually selected traits (Hau, 2007; Fusani, 2008; Ball and Balthazart, 2009). In combination with its effects on other fitness related traits (e.g., immune function, Segner et al., 2017), testosterone is thought to enforce the honesty of sexually selected traits (Ketterson and Nolan, Jr., 1999, Buchanan et al., 2001, Wingfield et al., 2001, Reed et al., 2006). Two prominent hypotheses have been proposed to explain how testosterone enforces the honesty of sexually selected traits: the Immunocompetence Handicap Hypothesis (Folstad and Karter, 1992) and the Oxidation Handicap Hypothesis (Alonso-Alvarez et al., 2007). The Immunocompetence Handicap Hypothesis proposes that sexually selected traits remain honest because of testosterone’s antagonistic effects on an individual’s immune function. Therefore, poor quality or low condition individuals cannot maintain high levels of circulating testosterone due its immunosuppressive effects (Folstad and Karter, 1992). A meta-analysis by Roberts et al. (2004) revealed weak support for this hypothesis. However, a meta-analysis by Foo et al. (2017) found that experimentally increasing testosterone results in suppression of both cell-mediated and humoral immunity. Foo et al. (2017) also found positive trends between multiple measures of immune function and naturally occurring levels of circulating testosterone. These results fit the predictions of the Immunocompetence Handicap Hypothesis because individuals naturally expressing high of testosterone represent high quality or high condition individuals that can invest in sexually selected traits without compromising their immune system (Peters, 2000). The Oxidation Handicap Hypothesis, on the other hand, states that sexually selected traits remain honest because testosterone increases an individual’s susceptibility and/or exposure to oxidative stress (Alonso-Alvarez et al., 2007). In other words, testosterone may influence an individual’s ability to protect or repair cellular machinery from oxidative damage (e.g., an individual’s antioxidant defenses) or testosterone may influence the rate that reactive oxygen species are produced (Alonso-Alvarez et al., 2007). Importantly, either one of these consequences may occur independent of the other. Of the few studies that have directly tested the Oxidation Handicap Hypothesis, some have found support (Mougeot et al., 2009; Hoogenboom et al., 2012) while results from others did not find support for this hypothesis (Isaksson et al., 2011; Casagrande et al., 2012; Taff and Freeman-Gallant, 2014; Baldo et al., 2015). Nonetheless, both hypotheses have primarily been tested using physiological measurements of oxidative stress and immunity, but less is known about the underlying molecular pathways. Given that sex steroids partly function by binding to intracellular receptors and acting as transcription factors (Ketterson and Nolan, Jr., 1999; Nelson, 2011), measuring the relationship between testosterone and transcription can shed light on the proximate pathways that testosterone influences.

Modern sequencing approaches, like RNA sequencing (RNA-seq), allow for comprehensive measurements of whole transcriptomes and the relative abundance of each transcript (Wang et al., 2009). This approach assesses coordinated, large-scale transcriptional responses rather than focusing on targeted candidate genes (e.g., via qPCR). RNA-seq approaches have been used to investigate the role of androgens on gene expression, particularly in the context of sex differences (Gao et al., 2015; Cox et al., 2017) and gonadal development (Monson et al., 2017; Zheng et al., 2019). Similarly, RNA-seq based studies have been crucial in
providing a more comprehensive understanding of the complex and dynamic immune and stress
responses (e.g., Barshis et al., 2013; Huang et al., 2013; Kim et al., 2018). In the context of mate
choice, measuring the relationship between testosterone and transcription can shed light on the
pathways that testosterone influences to potentially enforce the honesty of sexually selected traits
(e.g., immune or oxidative stress pathways). Therefore, our understanding of the pleiotropic
nature of testosterone is partly dependent upon our understanding of the impact of circulating
testosterone on the transcriptomic signatures of immunity and oxidative stress. To date, these
hypotheses have rarely been tested using genome scale approaches (Wenzel et al., 2013). In red
grouse (*Lagopus lagopus scoticus*), testosterone treatment had little effect on overall gene
expression in the liver and spleen but did result in the down-regulation of genes related to
immune function in caecal tissue (Wenzel et al., 2013). Given that Wenzel et al. (2013) used a
microarray-based approach, testing these handicap hypotheses using RNA-seq represents a more
modern, robust test as RNA-seq provides many advantages over microarray technologies,
including higher sensitivity and no hybridization biases (Wang et al., 2009).

Here, we use published RNA-seq datasets to further examine the effects of testosterone
on the transcriptome. Specifically, we re-analyze studies that compared gene expression between
testosterone-treatment and control subjects in two bird species: golden-collared manakin
(*Manacus vitellinus*) and Japanese quail (*Coturnix japonica*). Golden-collared manakin males
produce brightly colored plumage ornaments and engage in elaborate courtship behaviors during
the breeding season (Day et al., 2007). Importantly, previous work on the golden-collared
manakin experimentally blocked androgen receptors to show that the expression of male
reproductive behaviors is dependent upon the interaction between testosterone and the androgen
receptor (Day et al., 2007; Schlinger et al., 2013). Japanese quail males also produce brightly
colored cheek feathers to attract females (Hiyama et al., 2018). Castrating males influences the
color of a male’s cheek feathers and administering testosterone to castrated males causes cheek
patches of castrated males to match those of males that have not been castrated. In this study, we
use transcriptomic data from the foam gland of quail and muscular tissue of the golden-collared
manakin, tissues that are known to be express significant amounts of androgen receptors
(Adkins-Regan, 1999; Fuxjager et al., 2016). However, we re-analyze the data to explicitly test
the Immunocompetence Handicap Hypothesis and the Oxidation Handicap Hypothesis. We
constructed co-expression networks to identify gene networks that show correlated expression
patterns following testosterone treatment. If testosterone is immunosuppressive, then we predict
that testosterone treatment will cause consistent down-regulation (i.e. suppression) of genes with
annotated immune function in both species. Similarly, if testosterone influences an individual’s
susceptibility or exposure to oxidative stress, then we predict that testosterone treatment will
cause a decrease in the expression of genes with annotated functions in antioxidant protection
and/or an increase in genes that are expressed in response to oxidative stress. An important
caveat is that no support for either hypothesis does not exclude the possibility that these
pathways enforce the honestly of sexually selected traits independent of testosterone’s effects, as
has been suggested before (Metcalfe and Alonso-Alvarez, 2010; Weaver et al., 2017).

**Methods**

*Study Selection*
To identify studies of interest, we first performed a literature search on both Scopus and Google Scholar with the following search terms: “testosterone” AND “RNA-seq” or “transcriptome” or “transcriptomics”. This literature search produced 260 results. From this list of 260 studies, we retained RNA-seq studies that measured gene expression in adult males from both testosterone-manipulated and control groups. This process resulted in one study for re-analysis and we also identified an additional dataset by searching within NCBI’s Sequence Read Archive (Supplemental Figure 1). Fuxjager et al. (2016) experimentally increased testosterone in golden-collared manakins (*Manacus vitellinus*, “manakin”) and performed RNA-seq on pectoralis and scapulohumeralis caudalis tissue (n= 3 each testosterone and control for each tissue). Finseth and Harrison (2018) experimentally increased testosterone in Japanese quail (*Coturnix japonica*, “quail”) experiencing short days and performed RNA-seq on the foam gland (n=6 each testosterone and control).

**Data Re-analysis**

We downloaded the raw sequencing data from SRA with sratoolkit fastq-dump (quail: PRJNA397592; manakin: PRJNA297576) and adaptor trimmed all reads with Trim Galore! v0.3.8 (https://github.com/FelixKrueger/TrimGalore). We aligned trimmed reads to the respective reference genome (*M. vitellinus* v2, *C. japonica* v2) for each species with STAR v2.5.3 (Dobin et al., 2013) and quantified expression with htseq-count v0.6.0 (Anders et al., 2015), specifying strand ‘no’. We normalized counts to sequencing depth and variance stabilizing transformed counts with DEseq2 (Love et al., 2014). Transformed counts were visualized with a principal component analysis (PCA) using pcaExplorer v2.8.1 (Marini and Binder, 2016).

To test for the effect of testosterone treatment on transcription, we performed network analysis with the weighted gene co-expression network analysis (WGCNA) tool (Langfelder et al., 2011; Langfelder and Horvath, 2008). We created modules independently for each species with the following shared parameters: network type=signed, minimum module size=30, and module dissimilarity=0.2. We used β=12 for quail and β=18 for manakin, which represents the point the network reached scale free topology. We then tested for correlations between modules and testosterone treatment using a p<0.05 cutoff. We identified the hub genes of each module by selecting the top five genes with the highest module membership (MM) score.

To test the Immunocompetence and Oxidation Handicap Hypotheses, we performed ranked order gene ontology (GO) analyses with GOrilla (Eden et al., 2009, 2007). For each module, we ordered the gene list by descending MM scores and input this entire list into GOrilla. GOrilla then tests for enrichment and places greater weight on genes at the top of the list relative to the bottom. GO categories were significantly enriched if the qvalue < 0.05. To find support for the Immunocompetence Handicap Hypothesis, immune related GO categories (e.g., “immune system process”) had to be significantly enriched among down-regulated genes. To find support for the Oxidation Handicap Hypothesis, oxidative stress related GO categories had to be significantly enriched among up-regulated genes (e.g., “response to oxidative stress”) or down-regulated genes (e.g., “antioxidant activity”).

**Results**

**Overall Results**
After filtering, we used 13,509 manakin genes and 13,946 quail genes for PCA and WGCNA network construction. Testosterone treatment had pronounced effects on gene expression and individuals clustered by treatment in both comparisons (Figure 1).

WGCNA – Quail

WGCNA constructed 18 modules for quail, six of which were correlated with testosterone treatment (Supplemental Figure 2). The yellow module (925 genes, r=−0.74) and dark green module (88 genes, r=−0.67) were both strongly enriched for immune related GO categories (Table 1). The yellow module was primarily enriched for broad immune categories, e.g., “immune system process” and “immune response”, whereas the dark green module was primarily enriched for lymphocyte and leukocyte related categories. This represents a significant decrease in immune gene expression following treatment (Figure 2A). The yellow module hubs were SASH3, ITGB2, SLAMF8 (LOC107324444), TRAF3IP3, and EVI2A. The dark green hub genes were FBL, PIK3R6, STOML2, GPR157, and DNA14.

The black and purple modules were also negatively correlated with testosterone treatment and were enriched for translation and muscle process GO categories respectively. Lastly, we found two modules up-regulated following testosterone treatment. The turquoise module was the most strongly correlated with testosterone treatment (4423 genes, r=0.98). GO enrichment was largely driven by genes involved in the Golgi apparatus and endoplasmic reticulum functions (Supplemental Table 1). The green module (795 genes, r=0.61) was primarily enriched for broad metabolic activity and protein modification processes.

WGCNA – Manakin

WGCNA constructed 34 modules for manakin, 12 of which were correlated with testosterone treatment (Supplemental Figure 3). Seven modules were correlated with muscle type. None of these modules were also correlated with testosterone treatment, indicating no tissue specific response at the network level. Of the 12 modules, 7 were negatively correlated and 5 positively correlated. Like the quail, manakins also exhibited a significant decrease in immune gene expression following testosterone treatment (Figure 2B, Supplemental Table 2). The dark turquoise module (198 genes, r=−0.71) was strongly enriched for a broad range of immune related GO categories (Table 1). The dark turquoise hub genes were MHC1A (LOC108639055), INPPL1 (LOC103767762), CCL14 (LOC103758017), CCL3L (LOC103757995), and an uncharacterized non-coding RNA (LOC108640668).

The remaining negatively correlated modules were primarily enriched for metabolism (green, dark olive green), ribosomal components (dark red, pale turquoise), and mitochondria related categories (steel blue, pale turquoise). Among the positively correlated modules, we also found enrichment of cellular metabolism, catabolism, and mitochondrial related GO categories (Supplemental Table 2).

Discussion

In this study, we quantified transcriptional responses to experimentally increased circulating testosterone in two species of bird. Our gene network analysis revealed that both manakin and quail exhibit immunosuppression following testosterone treatment, supporting the
Immunocompetence Handicap Hypothesis. However, we did not find support for the Oxidation Handicap Hypothesis, as there was no enrichment of genes expressed related to oxidative damage, nor suppression of genes related to antioxidant defenses in either species. These results suggest that high levels of circulating testosterone can be costly to maintain partly due to their potential negative effects on an individual’s immune response and not the individual’s susceptibility or exposure to oxidative stress. Importantly, oxidative stress could still be involved in enforcing the costs of reproduction or sexually selected traits; however, our results suggest that this cost is not borne out via molecular pathways that are sensitive to testosterone, at least in the tissues and species examined here.

Our analyses revealed that transcriptomic immunosuppression was broad, encompassing aspects of both innate immunity (e.g., leukocyte activation and cytokine signaling) as well as adaptive immunity (e.g., antigen processing and presentation) across both species (Table 1). The observed effect of testosterone could occur through both genomic and non-genomic pathways, but regulation of the immune system by androgens receptors likely plays an important role (Trigunaite et al., 2015; Segner et al., 2017; Gubbels Bupp and Jorgensen, 2018). More specifically, while testosterone exposure and subsequent androgen receptor activity can promote innate immune cell differentiation and development, testosterone also reduces activity of these cells (Gubbels Bupp and Jorgensen, 2018). As such, the hub genes of the immune related modules highlight broad suppression of innate immune signaling (quail yellow: SASH3, SLAMF8, TRAF3IP3; manakin dark turquoise: INPPL1, CCL14, CCL3L, ncRNA; (Beer et al., 2005; Veillette, 2010; Dauphinee et al., 2013; Sokol and Luster, 2015; Zou et al., 2015; Thomas et al., 2017; Wang et al., 2018). Similarly, testosterone exposure had substantial effects on the regulation of the adaptive immune system. Testosterone exposure greatly reduces T cell activity (Lin et al., 2010; Kissick et al., 2014), which is a prominent signature in both quail (Supplemental Table 1) and manakin (Table 1). In addition to suppression of T cell activity in manakin, we also identified MHC class IA as a hub gene in the manakin dark turquoise module. MHC class IA binds and presents viral peptides to CD8+ T cells, which is a critical component of the adaptive immune response (Neefjes et al., 2011). Previous work has shown suppressive effects of testosterone on CD4+ T cells/MHC class IIB (Lin et al., 2010) and CD8+ T cells (Page et al., 2006). However, our study is the first to describe suppression of genes involved in T cell activity as well as MHC class I.

We were also interested in whether the changes in gene expression as a result of experimental testosterone treatment were consistent between manakin and quail. Despite evidence of immunosuppression in both species, the immune related gene networks are not preserved between the species (Supplemental Figure 4). These results suggest either a species specific and/or tissue specific response to testosterone treatment, both of which have previously been documented in transcriptomic data (Breschi et al., 2016). Given that muscle tissues used in the manakin study are very distinct from the foam gland tissue used in the quail study, it should not necessarily be surprising that the response to testosterone treatment was not preserved. Nonetheless, we identified these immunosuppression signatures in muscle and foam gland, tissues which are not traditionally studied in avian immunology (Rose, 1979; Schat et al., 2014). Thus, our results are likely conservative, and we may expect to see a stronger signature in immune tissues, such as broader suppression of adaptive immune response. This highlights the sensitivity of RNA-seq to detect functional signatures in non-traditional tissues (e.g., Louder et
al., 2018) In both species, testosterone is necessary to produce secondary sexual characteristics for mating (Schlinger et al., 2013, Hiyama et al., 2018). Our results detail the potential molecular pathways underlying the trade-off between the expression of sexually selected traits and immune function.

Given that we found strong support for immunosuppression in both studies, multiple experiments should be conducted to continue to broaden our understanding of testosterone’s immunosuppressive effects. First, studies should focus on performing testosterone manipulations and examining transcriptomic responses in a wider range of tissues and species. Moreover, studies should prioritize conducting experimental infections and/or immune challenges in combination with RNA-seq analyses to examine how transcriptomic signatures relate to immune function. Novel endocrine-based experiments, similar to (Goymann et al., 2015; Goymann and Flores Dávila, 2017), paired with RNA-seq analyses can also shed light on how acute changes in testosterone levels influence transcription over shorter timeframes. When possible, studies should also prioritize measuring testosterone’s effect on gene expression using a within-individual sampling approach as this allows for a more robust test of testosterone’s effect on gene expression (Williams, 2008). Overall, these integrative, mechanistic approaches will ultimately provide novel insights into the evolution of sexually selected traits.

Acknowledgements
We would like to thank Ignacio Moore, Dana Hawley, and Christopher Balakrishnan for providing helpful feedback on an earlier version of this manuscript.
Table and Figures

Table 1. GO enrichment for modules found in both species that support the Immunocompetence Handicap Hypothesis. The top 5 gene ontology (GO) categories are presented, along with FDR adjusted p-value and GOrilla enrichment score.

| GO ID               | Description                                      | FDR     | Enrichment |
|---------------------|--------------------------------------------------|---------|------------|
| **Quail, Yellow Module** |                                                  |         |            |
| GO:0002376          | immune system process                            | 3.11E-45| 4.53       |
| GO:0006955          | immune response                                  | 3.47E-35| 5.53       |
| GO:0002682          | regulation of immune system process              | 4.30E-35| 3.59       |
| GO:0002684          | positive regulation of immune system process     | 1.34E-32| 4.25       |
| GO:0046649          | lymphocyte activation                            | 1.08E-30| 11.1       |
| **Quail, Dark Green Module** |                                              |         |            |
| GO:0002684          | positive regulation of immune system process     | 4.09E-05| 1.86       |
| GO:1903706          | regulation of hemopoiesis                        | 5.89E-05| 2.17       |
| GO:0046649          | lymphocyte activation                            | 6.06E-05| 2.45       |
| GO:0038023          | signaling receptor activity                      | 6.38E-05| 1.83       |
| GO:0002682          | regulation of immune system process              | 6.42E-05| 1.61       |
| **Manakin, Dark Turquoise Module** |                                        |         |            |
| GO:0006955          | immune response                                  | 8.81E-10| 2.48       |
| GO:0046649          | lymphocyte activation                            | 3.29E-08| 7.44       |
| GO:0042110          | T cell activation                                | 3.57E-08| 10.58      |
| GO:0002376          | immune system process                            | 4.34E-08| 3.04       |
| GO:0002521          | leukocyte differentiation                         | 3.31E-07| 4.09       |
Figure 1. PCA of (A) manakin and (B) quail. Samples separate by treatment along PC3 for manakin and PC1 for quail. Each circle represents a sample and is color-coded by treatment. Manakin samples are labeled by muscle type. Ellipses represent 95% confidence intervals.
**Figure 2.** Expression heatmaps of the (A) Manakin Dark Turquoise Module and (B) Quail Yellow Module, which represent down-regulation of the immune system. Each column represents a sample color coded by treatment or muscle type. Each row represents a module gene. High expression is indicated by orange colors and low expression is represented by blue colors.
Adkins-Regan, E., 1999. Foam Produced by Male Coturnix Quail: What Is Its Function? The Auk 116, 184–193. https://doi.org/10.2307/4089465

Alonso-Alvarez, C., Bertrand, S., Faivre, B., Chastel, O., Sorci, G., 2007. Testosterone and oxidative stress: the oxidation handicap hypothesis. Proceedings of the Royal Society B: Biological Sciences 274, 819–825. https://doi.org/10.1098/rspb.2006.3764

Anders, S., Pyl, P.T., Huber, W., 2015. HTSeq-A Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166–169. https://doi.org/10.1093/bioinformatics/btu638

Andersson, M.B., 1994. Sexual selection, Monographs in behavior and ecology. Princeton University Press, Princeton, N.J.

Baldo, S., Mennill, D.J., Guindre-Parker, S., Gilchrist, H.G., Love, O.P., 2015. The Oxidative Cost of Acoustic Signals: Examining Steroid Versus Aerobic Activity Hypotheses in a Wild Bird. Ethology 121, 1081–1090. https://doi.org/10.1111/eth.12424

Ball, G.F., Balthazart, J., 2009. Neuroendocrine Regulation of Reproductive Behavior in Birds, in: Hormones, Brain and Behavior. Elsevier, pp. 855–897. https://doi.org/10.1016/B978-008088783-8.00025-5

Barshis, D.J., Ladner, J.T., Oliver, T.A., Seneca, F.O., Traylor-Knowles, N., Palumbi, S.R., 2013. Genomic basis for coral resilience to climate change. Proceedings of the National Academy of Sciences 110, 1387–1392. https://doi.org/10.1073/pnas.1210224110

Beer, S., Scheikl, T., Reis, B., Huser, N., Pfeffer, K., Holzmann, B., 2005. Impaired Immune Responses and Prolonged Allograft Survival in Sly1 Mutant Mice. Molecular and Cellular Biology 25, 9646–9660. https://doi.org/10.1128/MCB.25.21.9646-9660.2005

Breschi, A., Djebali, S., Gillis, J., Pervouchine, D.D., Dobin, A., Davis, C.A., Gingeras, T.R., Guigó, R., 2016. Gene-specific patterns of expression variation across organs and species. Genome Biology 17. https://doi.org/10.1186/s13059-016-1008-y

Buchanan, K.L., Evans, M.R., Goldsmith, A.R., Bryant, D.M., Rowe, L.V., 2001. Testosterone influences basal metabolic rate in male house sparrows: a new cost of dominance signalling? Proc. R. Soc. Lond. B 268, 1337–1344. https://doi.org/10.1098/rspb.2001.1669

Casagrande, S., Costantini, D., Groothuis, T.G.G., 2012. Interaction between sexual steroids and immune response in affecting oxidative status of birds. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 163, 296–301. https://doi.org/10.1016/j.cbpa.2012.07.018

Cox, R.M., Cox, C.L., McGlothlin, J.W., Card, D.C., Andrew, A.L., Castoe, T.A., 2017. Hormonally Mediated Increases in Sex-Biased Gene Expression Accompany the Breakdown of Between-Sex Genetic Correlations in a Sexually Dimorphic Lizard. The American Naturalist 189, 315–332. https://doi.org/10.1086/690105

Dauphinee, S.M., Clayton, A., Hussainkhel, A., Yang, C., Park, Y.-J., Fuller, M.E., Blonder, J., Veenstra, T.D., Karsan, A., 2013. SASH1 Is a Scaffold Molecule in Endothelial TLR4 Signaling. The Journal of Immunology 191, 892–901. https://doi.org/10.4049/jimmunol.1200583

Day, L.B., Fusani, L., Hernandez, E., Billo, T.J., Sheldon, K.S., Wise, P.M., Schlinger, B.A., 2007. Testosterone and its effects on courtship in golden-collared manakins (Manacus vitellinus): Seasonal, sex, and age differences. Hormones and Behavior 51, 69–76. https://doi.org/10.1016/j.yhbeh.2006.08.006
Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., Gingeras, T.R., 2013. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–21.

Eden, E., Lipson, D., Yogev, S., Yakhini, Z., 2007. Discovering Motifs in Ranked Lists of DNA Sequences. PLOS Computational Biology 3, e39–e39.

Eden, E., Navon, R., Steinfeld, I., Lipson, D., Yakhini, Z., 2009. GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. BMC Bioinformatics 10, 48–48. https://doi.org/10.1186/1471-2105-10-48

Finseth, F.R., Harrison, R.G., 2018. Genes Integral to the Reproductive Function of Male Reproductive Tissues Drive Heterogeneity in Evolutionary Rates in Japanese Quail. G3: Genes|Genomes|Genetics 8, 39–51. https://doi.org/10.1534/g3.117.300095

Folstad, I., Karter, A.J., 1992. Parasites, Bright Males, and the Immunocompetence Handicap. The American Naturalist 139, 603–622. https://doi.org/10.1086/285346

Foo, Y.Z., Nakagawa, S., Rhodes, G., Simmons, L.W., 2017. The effects of sex hormones on immune function: a meta-analysis: Sex hormones and immune function. Biological Reviews 92, 551–571. https://doi.org/10.1111/brv.12243

Fusani, L., 2008. Testosterone control of male courtship in birds. Hormones and Behavior 54, 227–233. https://doi.org/10.1016/j.yhbeh.2008.04.004

Fuxjager, M.J., Lee, J.-H., Chan, T.-M., Bahn, J.H., Chew, J.G., Xiao, X., Schlinger, B.A., 2016. Research Resource: Hormones, Genes, and Athleticism: Effect of Androgens on the Avian Muscular Transcriptome. Molecular Endocrinology 30, 254–271. https://doi.org/10.1210/me.2015-1270

Gao, J., Liu, S., Zhang, Y., Yang, Y., Yuan, C., Chen, S., Wang, Z., 2015. Effects of 17 α-methyltestosterone on transcriptome, gonadal histology and sex steroid hormones in rare minnow Gobiocypris rarus. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 15, 20–27. https://doi.org/10.1016/j.cbd.2015.05.001

Goymann, W., Flores Dávila, P., 2017. Acute peaks of testosterone suppress paternal care: evidence from individual hormonal reaction norms. Proceedings of the Royal Society B: Biological Sciences 284, 20170632. https://doi.org/10.1098/rspb.2017.0632

Goymann, W., Villavicencio, C.P., Apfelbeck, B., 2015. Does a short-term increase in testosterone affect the intensity or persistence of territorial aggression? — An approach using an individual’s hormonal reactive scope to study hormonal effects on behavior. Physiology & Behavior 149, 310–316. https://doi.org/10.1016/j.physbeh.2015.06.029

Grafen, A., 1990. Biological signals as handicaps. Journal of Theoretical Biology 144, 517–546. https://doi.org/10.1016/S0022-5193(05)80088-8

Gubbels Bupp, M.R., Jorgensen, T.N., 2018. Androgen-Induced Immunosuppression. Frontiers in Immunology 9. https://doi.org/10.3389/fimmu.2018.00794

Hau, M., 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. BioEssays 29, 133–144. https://doi.org/10.1002/bies.20524

Hiyama, G., Mizushima, S., Matsuzaki, M., Tobari, Y., Choi, J.-H., Ono, T., Tsudzuki, M., Makino, S., Tamiya, G., Tsukahara, N., Sugita, S., Sasanami, T., 2018. Female Japanese quail visually differentiate testosterone-dependent male attractiveness for mating preferences. Scientific Reports 8. https://doi.org/10.1038/s41598-018-28368-z

Hoogenboom, M.O., Metcalfe, N.B., Groothuis, T.G.G., de Vries, B., Costantini, D., 2012. Relationship between oxidative stress and circulating testosterone and cortisol in pre-spawning female brown trout. Comparative Biochemistry and Physiology Part A:
Molecular & Integrative Physiology 163, 379–387.
https://doi.org/10.1016/j.cbpa.2012.07.002

Huang, Y., Li, Y., Burt, D.W., Chen, H., Zhang, Y., Qian, W., Kim, H., Gan, S., Zhao, Yiqiang, Li, J., Yi, K., Feng, H., Zhu, P., Li, B., Liu, Q., Fairley, S., Magor, K.E., Du, Z., Hu, X., Goodman, L., Tafer, H., Vignal, A., Lee, T., Kim, K.-W., Sheng, Z., An, Y., Searle, S., Herrero, J., Groenen, M.A.M., Crooijmans, R.P.M.A., Faraut, T., Cai, Q., Webster, R.G., Aldridge, J.R., Warren, W.C., Bartschat, S., Kehr, S., Marz, M., Stadler, P.F., Smith, J., Kraus, R.H.S., Zhao, Yaofeng, Ren, L., Fei, J., Morisson, M., Kaiser, P., Griffin, D.K., Rao, M., Pitel, F., Wang, J., Li, N., 2013. The duck genome and transcriptome provide insight into an avian influenza virus reservoir species. Nature Genetics 45, 776–783.
https://doi.org/10.1038/ng.2657

Isaksson, C., While, G., McEvoy, J., van de Crommenacker, J., Olsson, M., Groothuis, T., Komdeur, J., Wapstra, E., 2011. Aggression, but not testosterone, is associated to oxidative status in a free-living vertebrate. Behaviour 148, 713–731.
https://doi.org/10.1163/000579511X574204

Ketterson, E.D., Nolan, Jr., V., 1999. Adaptation, Exaptation, and Constraint: A Hormonal Perspective. The American Naturalist 154, S4–S25. https://doi.org/10.1086/303280

Kim, J., Kim, H., Son, C., 2018. Tissue-Specific Profiling of Oxidative Stress-Associated Transcriptome in a Healthy Mouse Model. International Journal of Molecular Sciences 19, 3174. https://doi.org/10.3390/ijms19103174

Kissick, H.T., Sanda, M.G., Dunn, L.K., Pellegrini, K.L., On, S.T., Noel, J.K., Arredouani, M.S., 2014. Androgens alter T-cell immunity by inhibiting T-helper 1 differentiation. Proceedings of the National Academy of Sciences 111, 9887–9892.
https://doi.org/10.1073/pnas.1402468111

Kokko, H., Brooks, R., Jennions, M.D., Morley, J., 2003. The evolution of mate choice and mating biases. Proc. R. Soc. Lond. B 270, 653–664. https://doi.org/10.1098/rspb.2002.2235

Langfelder, P., Horvath, S., 2008. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9, 559–559. https://doi.org/10.1186/1471-2105-9-559

Langfelder, P., Luo, R., Oldham, M.C., Horvath, S., 2011. Is My Network Module Preserved and Reproducible? PLoS Computational Biology 7, e1001057. https://doi.org/10.1371/journal.pcbi.1001057

Lin, A.A., Wojciechowski, S.E., Hildeman, D.A., 2010. Androgens suppress antigen-specific T cell responses and IFN-γ production during intracranial LCMV infection. Journal of Neuroimmunology 226, 8–19. https://doi.org/10.1016/j.jneuroim.2010.05.026

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biology 15, 550–550.
https://doi.org/10.1186/s13059-014-0550-8

Marini, F., Binder, H., 2016. Development of Applications for Interactive and Reproducible Research: a Case Study. Genomics and Computational Biology 3, 39. https://doi.org/10.18547/gcb.2017.vol3.iss1.e39

Metcalfe, N.B., Alonso-Alvarez, C., 2010. Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death: Oxidative stress as a life-history constraint. Functional Ecology 24, 984–996. https://doi.org/10.1111/j.1365-2435.2010.01750.x
Monson, C., Forsgren, K., Goetz, G., Harding, L., Swanson, P., Young, G., 2017. A teleost androgen promotes development of primary ovarian follicles in coho salmon and rapidly alters the ovarian transcriptome†. Biology of Reproduction 97, 731–745. https://doi.org/10.1093/biolre/iox124

Mougeot, F., Martínez-Padilla, J., Webster, L.M.I., Blount, J.D., Pérez-Rodríguez, L., Piertney, S.B., 2009. Honest sexual signalling mediated by parasite and testosterone effects on oxidative balance. Proceedings of the Royal Society B: Biological Sciences 276, 1093–1100. https://doi.org/10.1098/rspb.2008.1570

Neefjes, J., Jongsma, M.L.M., Paul, P., Bakke, O., 2011. Towards a systems understanding of MHC class I and MHC class II antigen presentation. Nature Reviews Immunology 11, 823–836. https://doi.org/10.1038/nri3084

Nelson, R.J., 2011. An introduction to behavioral endocrinology, 4th ed. ed. Sinauer Associates, Sunderland, MA.

Page, S.T., Plymate, S.R., Bremner, W.J., Matsumoto, A.M., Hess, D.L., Lin, D.W., Amory, J.K., Nelson, P.S., Wu, J.D., 2006. Effect of medical castration on CD4 + CD25 + T cells, CD8 + T cell IFN-γ expression, and NK cells: a physiological role for testosterone and/or its metabolites. American Journal of Physiology-Endocrinology and Metabolism 290, E856–E863. https://doi.org/10.1152/ajpendo.00484.2005

Peters, A., 2000. Testosterone treatment is immunosuppressive in superb fairy–wrens, yet free–living males with high testosterone are more immunocompetent. Proceedings of the Royal Society of London. Series B: Biological Sciences 267, 883–889. https://doi.org/10.1098/rspb.2000.1085

Reed, W.L., Clark, M.E., Parker, P.G., Raouf, S.A., Arguedas, N., Monk, D.S., Snajdr, E., Nolan Jr., V., Ketterson, E.D., 2006. Physiological Effects on Demography: A Long-Term Experimental Study of Testosterone’s Effects on Fitness. The American Naturalist 167, 667–683. https://doi.org/10.1086/503054

Roberts, M.L., Buchanan, K.L., Evans, M.R., 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. Animal Behaviour 68, 227–239. https://doi.org/10.1016/j.anbehav.2004.05.001

Rose, M.E., 1979. The Immune System in Birds. Journal of the Royal Society of Medicine 72, 701–705. https://doi.org/10.1177/01407687907200914

Schat, K., Kaspers, B., Kaiser, P. (Eds.), 2014. Avian Immunology. Elsevier.

Schlinger, B.A., Barske, J., Day, L., Fusani, L., Fuxjager, M.J., 2013. Hormones and the neuromuscular control of courtship in the golden-collared manakin (Manacus vitellinus). Frontiers in Neuroendocrinology 34, 143–156. https://doi.org/10.1016/j.yfrne.2013.04.001

Segner, H., Verburg-van Kemenade, B.M.L., Chadzinska, M., 2017. The immunomodulatory role of the hypothalamus–pituitary–gonad axis: Proximate mechanism for reproduction–immune trade offs? Developmental & Comparative Immunology 66, 43–60. https://doi.org/10.1016/j.dci.2016.07.004

Sokol, C.L., Luster, A.D., 2015. The Chemokine System in Innate Immunity. Cold Spring Harbor Perspectives in Biology 7, a016303. https://doi.org/10.1101/cshperspect.a016303

Stearns, S.C., 1992. The evolution of life histories. Oxford University Press, Oxford ; New York.
Taff, C.C., Freeman-Gallant, C.R., 2014. An experimental test of the testosterone mediated oxidation handicap hypothesis in a wild bird. Hormones and Behavior 66, 276–282. https://doi.org/10.1016/j.yhbeh.2014.05.006

Thomas, M.P., Erneux, C., Potter, B.V.L., 2017. SHIP2: Structure, Function and Inhibition. ChemBioChem 18, 233–247. https://doi.org/10.1002/cbic.201600541

Trigunaite, A., Dimo, J., Jørgensen, T.N., 2015. Suppressive effects of androgens on the immune system. Cellular Immunology 294, 87–94. https://doi.org/10.1016/j.cellimm.2015.02.004

Veillette, A., 2010. SLAM-Family Receptors: Immune Regulators with or without SAP-Family Adaptors. Cold Spring Harbor Perspectives in Biology 2, a002469–a002469. https://doi.org/10.1101/cshperspect.a002469

Wang, M., Jiang, S., Wu, W., Yu, F., Chang, W., Li, P., Wang, K., 2018. Non-coding RNAs Function as Immune Regulators in Teleost Fish. Frontiers in Immunology 9. https://doi.org/10.3389/fimmu.2018.02801

Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nature Reviews Genetics 10, 57–63. https://doi.org/10.1038/nrg2484

Weaver, R.J., Koch, R.E., Hill, G.E., 2017. What maintains signal honesty in animal colour displays used in mate choice? Phil. Trans. R. Soc. B 372, 20160343. https://doi.org/10.1098/rstb.2016.0343

Wenzel, M.A., Webster, L.M.I., Paterson, S., Mougeot, F., Martínez-Padilla, J., Pierney, S.B., 2013. A transcriptomic investigation of handicap models in sexual selection. Behavioral Ecology and Sociobiology 67, 221–234. https://doi.org/10.1007/s00265-012-1442-0

Williams, T.D., 2008. Individual variation in endocrine systems: moving beyond the ‘tyranny of the Golden Mean.’ Phil. Trans. R. Soc. B 363, 1687–1698. https://doi.org/10.1098/rstb.2007.0003

Wingfield, J.C., Lynn, S.E., Soma, K.K., 2001. Avoiding the ‘Costs’ of Testosterone: Ecological Bases of Hormone-Behavior Interactions. Brain Behav Evol 57, 239–251. https://doi.org/10.1159/000047243

Zahavi, A., 1975. Mate selection—A selection for a handicap. Journal of Theoretical Biology 53, 205–214. https://doi.org/10.1016/0022-5193(75)90111-3

Zera, A.J., Harshman, L.G., 2001. The Physiology of Life History Trade-Offs in Animals. Annual Review of Ecology and Systematics 32, 95–126. https://doi.org/10.1146/annurev.ecolsys.32.081501.114006

Zheng, Y., Yuan, J., Meng, S., Chen, J., Gu, Z., 2019. Testicular transcriptome alterations in zebrafish (Danio rerio) exposure to 17β-estradiol. Chemosphere 218, 14–25. https://doi.org/10.1016/j.chemosphere.2018.11.092

Zou, Q., Jin, J., Xiao, Y., Hu, H., Zhou, X., Jie, Z., Xie, X., Li, J.Y.H., Cheng, X., Sun, S.-C., 2015. T cell development involves TRAF3IP3-mediated ERK signaling in the Golgi. The Journal of Experimental Medicine 212, 1323–1336. https://doi.org/10.1084/jem.20150110