The Cost of Being Sexy

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The long quest to understand male sexual traits, testosterone, and immunocompetence

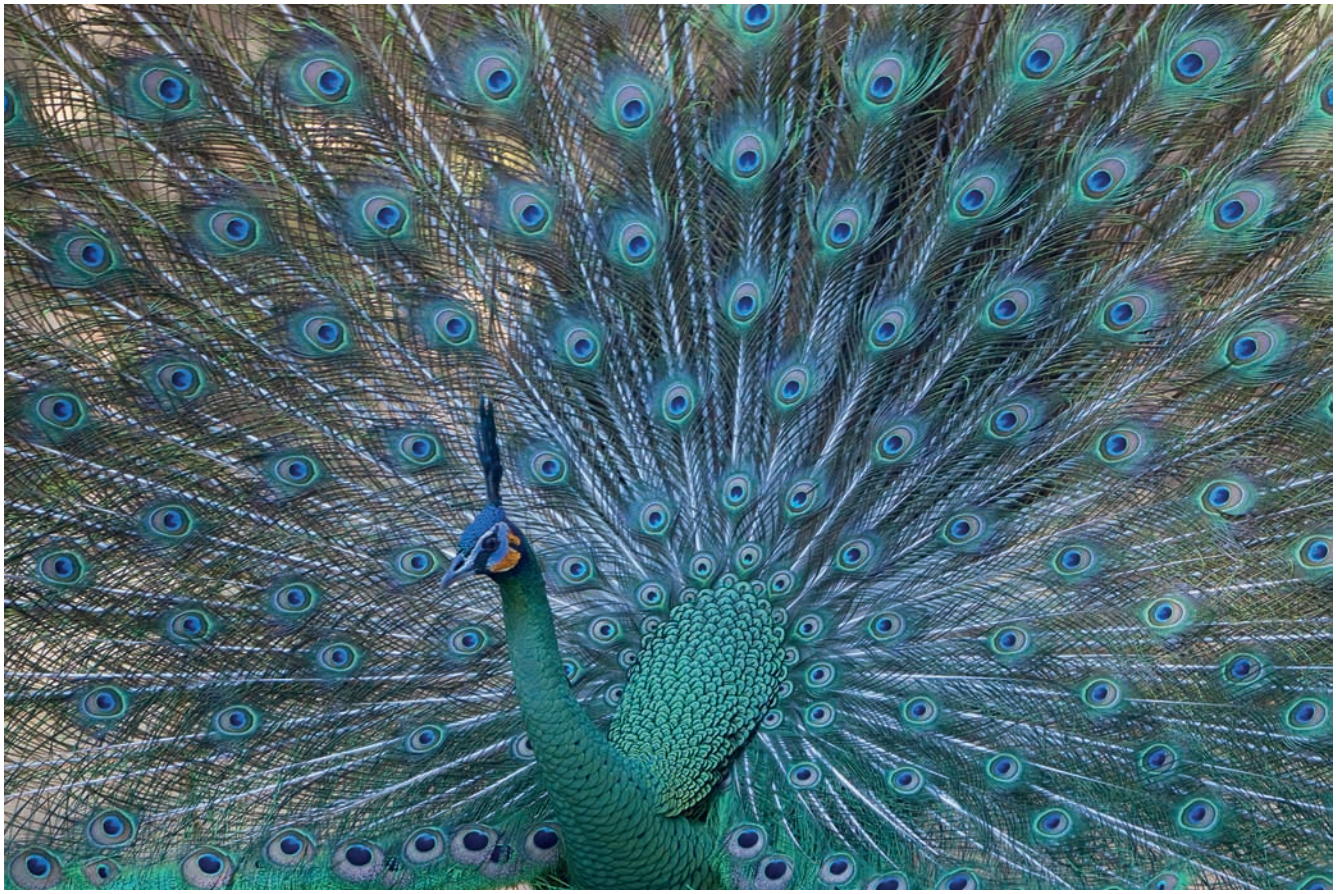
Cape ground squirrels have captured Jane Waterman's fascination for the last 30 years. "They're so cool," effuses Waterman. Studying them in South Africa, where these beguiling, furry mammals breed year round, she has revealed a treasure trove of surprises. Extremely social, females live in family groups with their young. Males disperse from their family group at about 3 years old, joining bachelor groups. In these all-male groups, males sleep together, groom one another, and move together through the veld. Males have no dominance hierarchy and defend no territories, and it is rare to see any sort of aggressive behavior, explains Waterman. But on the day when a female enters estrus, which lasts about 3 hours, an average of 11 males show up to try to breed. Winners in this mating game are typically older, in better body condition, and "well endowed" with big testes and more sperm, she says. Big testes, theory holds, are usually supported with an abundance of testosterone. But Waterman has not found their testosterone levels higher than those in a lot of other less-endowed species.

Studied by Jane Waterman, male Cape ground squirrels (*Xerus inauris*) found in South Africa are "well endowed" with big testes. Males also have more ectoparasites than females. Contrary to research in other animals, recent work showed no impact of experimentally elevating testosterone on immunity in this species. Photograph: Jane Waterman.

This intrigued her. It seemed to Waterman that to support big testes, males had to be able to keep testosterone going year round. In these squirrels, she also found more ectoparasites on males than on females—not only when males are wide-ranging adults but also when they are homebody juveniles. Her hunch was that an influx of testosterone drives a reduced male ability to fend off ectoparasites, espousing an idea known as the immunocompetence handicap hypothesis (ICHH). It is a hypothesis that she, with a graduate student, has recently tested experimentally.

Waterman's test of the ICHH follows on the heels of hundreds of studies since the idea was first articulated in *The American Naturalist* by Ivar Folstad and Andrew Karter in 1992. The ICHH proposes that elaborate male ornaments or signs of vigor are mediated at least in part by testosterone but that testosterone is costly in terms of compromising immune function. The ICHH is an influential hypothesis tracing its lineage back to Darwin's first musings about the tail of the peacock, the antlers of deer, and other exaggerated male traits that could attract the attention of females but might be costly for survival. For scientists who had long thought about sexual selection from a theoretical

BioScience 68: 393–399.
doi:10.1093/biosci/biy043



The mesmerizing eye spots, bright colors, and iridescence of the over-the-top tail of the peacock caused much consternation for Charles Darwin. Peacocks and other highly decorated and armored males contributed to his thinking and the eventual publication of his theory of sexual selection. Photograph: J. J. Harrison (jjharrison89@facebook.com).

standpoint, here was a potential mechanism to explain it. The duality of testosterone—sexy but costly—suggested authors Folstad and Karter, provides a cue to choosy females as to which males cope with this burden best.

Roots of the hypothesis

The roots of ICHH go back to Darwin, who already understood that being sexy could be costly. Among the many things that piqued his curiosity when it came to sex, the tail of the peacock puzzled Charles Darwin. In correspondence with American botanist colleague Asa Gray, Darwin wrote that "the sight of a feather in a peacock's tail, whenever I gaze at it, makes me sick!" His comments were tongue in cheek, but his bewilderment was real. Such pretty, exaggerated peacock plumage might be helpful in attracting the attentions of females, he surmised, but is totally impractical for a quick getaway from would-be predators, leading to an evolutionarily selective tug of war. Darwin developed these and other thoughts into theory in his 1871 book, *The Descent of Man, and Selection in Relation to Sex*. Nearly 150 years later, scientists are still fascinated by sexual selection.

Important stepping-stones between Darwin and the ICHH include the handicap principle proposed in 1975 by Israeli evolutionary biologist Amotz Zahavi. "An individual with a well developed sexually selected character [such as a peacock's flashy tail] is an individual which has survived a test. A female which could discriminate between a male possessing a sexually selected character, from one without it, can discriminate between a male which has passed a test and one which has not been tested," Zahavi wrote. His idea of male "honest signals"—bright feathers signaling good health, for example —has been much debated. Others have shown that dishonest signals—attractive traits that can deceive the eye of the beholder into believing he is healthy when he is not—can drive trait evolution, too. Nevertheless, Zahavi's idea of honest signals was highly influential in the study of sexual selection.

Zahavi's idea was expanded on by William Hamilton and Marlene Zuk in 1982. They proposed that health and resistance to parasites could be indicators of individual condition and possible drivers of the evolution of secondary sexual characteristics such as colorful plumage. Brighter plumage, they suggested, could be a reliable

signal to females of a male's "good genes" for a superior immune system to defend against parasites.

Entering the fray of what had already become a thriving research area, Folstad and Karter in 1992 reasoned that although a high level of testosterone "confers the benefits of exaggerated secondary sexual development and potentially increased mating success, such a condition simultaneously impairs the functioning of the immune system. This double-edged sword creates a real and potential physiological dilemma for males." Their hypothesis, referring specifically to the ability of the immune system to avoid or fight parasitic infections, provided an appealing proximate mechanism. Perhaps testosterone—good for reproduction, bad for immune function—was a mediator of the catch-22 of natural versus sexual selection.

The immunocompetence handicap hypothesis and a modified form of the hypothesis were tested by Kate Buchanan and colleagues in zebra finches, the lab rats of the bird physiology world. Her team found that high plasma levels of corticosterone are not immunosuppressive but rather immunoenhancing in the presence of high levels of plasma testosterone. Photograph: Peripitus.

Hypothesis testing

Kate Buchanan, now at Deakin University, in Victoria, Australia, began her postdoctoral studies a few years after the ICHH was published. At that time, testing links between immune systems and the development of sexual traits was "one of the hot areas of behavioral ecology," she says. "Everyone wanted to know, 'Why do you have parrots with bright colors? Why do you have males with long tails? Why do you have these amazing songs that have developed? What explains why females choose these traits that become elaborated… and why is this an evolutionarily stable strategy?'" Buchanan tested the hypothesis in zebra finches and quails. But she was not alone.

Intrigued by the possibility of the ICHH to examine the costs of sexual signaling, a flurry of experimental, observational, and correlational lab and field studies emerged. According to Web of Science, since its publication, Folstad and Karter's paper has been cited more than 1700 times.

One early test of the ICHH on a free-living animal was conducted by Spanish and Swedish researchers on a population of Mediterranean ground-dwelling lizards. In *Psammodromus algirus*, 29 males were captured, counted for ticks, and assigned to an experimental or control group receiving a subcutaneous implant of a silastic tube that was either empty or filled with a dose of testosterone propionate. On later recapture, ticks were again counted, and blood was collected from each male to perform counts of red and white blood cells. At the end of the experiment, more ticks were found on testosterone-treated males, providing support for the ICHH.

In other reptiles, studies were conducted on tree lizards, fence lizards, anoles, and Galapagos iguanas. In mammals, the hypothesis was tested on lab mice, hamsters, and white-tailed deer. In birds, researchers tested the hypothesis on a variety of captive and free-living species, including pheasants, penguins, house sparrows, honeyeaters, chickens, and starlings. Many studies were conducted on juncos implanted with testosterone or a sham control. Results indicated "that prolonged elevation of testosterone suppresses antibody production in captive males and cell-mediated immunity in wild males," wrote Indiana University's Ellen Ketterson and colleagues, who concluded that testosterone-treated males might be more susceptible to disease or parasitic infection.

Researchers know that testosterone is important in controlling the production of sexual traits and that its level goes up and down in key life periods, explains Buchanan. "There is good evidence that testosterone is immunosuppressant at least some of the time," she says. In her own studies, Buchanan tested the original and a modified version of the hypothesis. That modified version, one of many that has been proposed, suggests that testosterone is immunosuppressive only indirectly via increasing production of corticosterone. What Buchanan found in her 2007 zebra finch study was not immunosuppression but immune enhancement, something many other studies

were finding, too. Her results contradicted the ICHH central assumption that testosterone is obligately immunosuppressive.

But in the early days of test-driving the hypothesis, "evolutionary ecologists hadn't had much practice at wrestling with the immune system, and of course the immune system is pretty complicated," Buchanan says. As more and more studies emerged, mirroring the diversity of organisms studied, the results testing the premise of the ICHH were mixed.

Buchanan was part of a 2004 meta-analysis of the ICHH, examining 36 studies of reptiles, birds, and mammals. They revealed a significant immunosuppressive effect of testosterone, but the effect disappeared after controlling for multiple studies on the same species. In reptiles, they found a trend for testosterone to increase ectoparasite abundance—more testosterone meant more ticks. But overall, they concluded, "There is at best weak evidence that testosterone directly influences immune function in males according to the ICHH."

Zuleyma Tang-Martinez and Stan Braude argued similarly at the 2017 Animal Behaviour meeting in Toronto, Canada. Tang-Martinez, professor emerita of biology at the University of Missouri-St. Louis, recalls much interest in ICHH when the idea first emerged. The hypothesis fascinated her, but "there was always a little question in the back of my mind about whether people were jumping on the bandwagon too quickly," she says. That lingering doubt remains.

Part of the ambiguity in results, she suggests, stems from the assumption that it is relatively easy to measure immunocompetence. Studies testing the ICHH have measured many different immune indicators. Some measure a response to immune challenges an animal might be exposed to naturally, such as infection by common pathogens or seeding wounds with bacteria. Other tests have used exposures such as invasively introduced antigens from limpets and plants that an animal might never otherwise encounter. Although some argue that novel exposures are a good way to measure immune response, others argue that the evolutionary novelty might render reaction to them uninformative.

Dark-eyed juncos (*Junco hyemalis*) are the Olympians of testosterone implant studies, with many studies conducted by Ellen Ketterson and colleagues. In juncos, prolonged elevation of testosterone suppresses antibody production in captive males and suppresses cell-mediated immunity in wild males. Photograph: Dick Daniels (http://carolinabirds.org).

Beyond the wide variety of immune tests used, other factors not always accounted for might matter, too. Testosterone–immune relationships, it turns out, can change with breeding condition, food availability, body condition, seasonality, life history, social system, mating system, dominance status, and stress levels. Results from the field under natural conditions have often been different from lab results.

But some new evidence bolsters support for the hypothesis. A 2016 meta-analysis conducted by Yong Zhi Foo, at the University of Western Australia, and colleagues reviewed an expanded list of studies. Within an overall look at 122 studies of both testosterone and estrogen, they analyzed 94 studies examining testosterone. The researchers broke down the analyses into 66 experimental and 28 correlational studies, also accounting for mating system, natural versus lab populations, possible publication bias, phylogeny, hormone dosage, whether the endogenous production of hormones was controlled by measures such as castration, and what type of immune response was measured.

Noting lots of variability in the data set, they nevertheless found support for testosterone-suppressing immune function, finding an effect size almost twice that of the previous meta-analysis. Buchanan finds their result "a more valid conclusion than our initial look-see," she says. In contrast to the experimental studies examined, Foo and coauthors did not find a significant overall correlation between testosterone and immune function in correlational studies. From this they conclude, "correlational studies are not ideal for testing the effect of testosterone on immune function."

On this point, they cite earlier work (2006) on signaling theory by Michigan State University's Thomas Getty. He explains that under the ICHH, the expectation is that the more testosterone there is associated with some signal, the lower the immunocompetence, representing a negative trade-off within individuals. However, he explains, that inverse relationship is not the only possibility. A positive correlation across individuals can result, too, an idea that can be explained with an analogy to conspicuous consumption (wealth signaling) in humans.

As Getty explains, rich people can spend more money on luxury cars than poor people spend on economy models but still have more money left in the bank. There are negative trade-offs within both classes—the more money spent on a car, the less there is in the bank. But the two classes are on different trade-off curves. Similarly, in thinking about the ICHH in animals, individuals in better health—with good genes, good luck, or better nurture—have high immunocompetence to begin with. In contrast, individuals in poor health—with bad genes, bad luck, or worse nurture—have low immunocompetence to begin with. For both classes of individuals, as they elevate testosterone, their immunocompetence declines. But high-quality individuals can have more testosterone *and* higher immunocompetence, leading to a positive instead of inverse relationship between testosterone and immune function.

Foo also notes that recent evidence points to the possibility that although testosterone can influence immune function, immune function can influence testosterone, too. In immune activation studies by University of Groningen biologist Jelle Boonekamp and colleagues, mice, rabbits, rats, sheep, collared flycatchers, and chickens that were given immune challenges, including malaria, schistosoma, bronchitis, and mites, experienced measurably suppressed testosterone levels.

This reverse causality is being hinted at in the wild, too. Back in South Africa with the Cape ground squirrels, who have so much male investment in their gonads, Waterman was curious to know how this influenced immune function. "Like most people, I just assumed the ICHH was supported there—that they had high testosterone that would suppress their immune system." In fact, her study, recently submitted to the *Journal of Experimental Biology*, showed no impact of experimentally elevating testosterone on immunity. But intriguingly, when her graduate student caused an immune reaction in the squirrels, testosterone dropped significantly. "We don't think it's testosterone suppressing immunity in this species—it's immunity suppressing testosterone," says Waterman.

Greg Demas, now at Indiana University Bloomington, was similarly surprised by his doctoral research experiments on seasonal changes in immune function. Studying deer mice in the lab at Johns Hopkins University, in short days of winter, he found that the mice would turn off their reproductive systems, and as they did this, their immune systems ramped up. His dissertation was aimed at figuring out the mechanism. "One of the obvious mechanisms was, 'T [testosterone] is immunosuppressant,' because you turn off your gonads, and you release yourself from the immunosuppressant effects of T." But that is not what he found. Instead, he found that a gonadectomy to reduce testosterone had no link to immune function.

In reflecting on the research field as a whole and the inconsistent results of testing the ICHH, Demas says, "The idea of testosterone being immunosuppressant has become dogmatic." Often, he observes, if the data analyzed to test the ICHH do not support it, alternative hypotheses are suggested to explain around it rather than accepting that testosterone is not always immunosuppressant.

Indeed, many researchers agree that a problematic element of the reductionist ICHH is that it oversimplifies

These sand lizards (*Lacerta agilis*), studied by Mats Olsson, Erik Wapstra, and colleagues, were one of the many lizard species in which the ICHH was tested by examining number of ectoparasites (e.g., ticks) versus testosterone levels. Photographs: Erik Wapstra.

immunocompetence and immunosuppression. "We can't treat immunity as this monolithic entity," says Tierney Lorenz, professor of health psychology at the University of North Carolina at Charlotte. She also points out that it is not only males that have testosterone. Although researchers have come to view it as a hormone with proreproductive but immunosuppressive effects, in her studies on the effects of testosterone on immunity in women, she says, "We've found it a great deal more complex than that." Tierney has found that testosterone has an immune-boosting effect during some phases of the menstrual cycle, particularly for sexually active versus abstinent women.

Studied by Greg Demas, at Indiana University, these Siberian hamsters have gonads that change size with the seasons. Reproductively active hamsters (in conditions of long days) have high testosterone and stronger immune responses than gonadally regressed short-day hamsters, which have undetectable testosterone and lower measures of immune response. Photographs: Greg Demas.

Complications, alternative hypotheses, and future directions

When it comes to sexual signaling of elaborate male traits, the literature on testosterone is "spectacularly confusing," says Rebecca Adrian (formerly Koch), a postdoctoral research fellow at Monash University, in Melbourne, Australia, who has studied sexual signaling in the colorful displays of canaries and other birds.

Adrian views testosterone as an intermediate and clearly important signaling molecule but not necessarily the mediator in crosstalk with the immune system. She has been part of a team investigating an alternative possibility that mitochondria—as signaling hubs and metabolic regulators of immune function—might be the shared pathway linking ornament production and individual condition. The idea is new to animal behavior but not new to the biomedical field. Another variant of the ICHH that researchers are beginning to test, such as in recent work on free-living yellowthroat warblers, is the testosterone-mediated oxidation handicap hypothesis—the idea that testosterone generates oxidative stress. Oxidative stress is caused by increased production of unstable, highly reactive oxygen species that buzz around haphazardly until they smack into and damage cell components, including DNA, lipid membranes, and proteins, sometimes causing cell death.

Another explanation for variability in testosterone–immune relationships is that the immune system may not actually be ramping down or ramping up as a whole but rather reallocating resources to where they are needed. Stan Braude, a former postdoc with Tang-Martinez, now at Washington University, in St. Louis, is unconvinced that an organism's physiology would ever compromise its own immunity. White blood cells responding to wounding might move from blood to tissue, for example—something Demas explains with a military metaphor. If you have soldiers concentrated in the main barracks and suddenly a war breaks out, they are going to move out to the front. If you sample the barracks at that point, you are not going to find many soldiers. Assuming the ICHH is true, that drop in numbers would be interpreted as immune suppression. By contrast, the immune redistribution hypothesis recognizes that the soldiers are still there; they have just moved around. By this logic, testosterone and other hormones could be modulators of immune migration.

Further reading.

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Debate will probably continue over whether the ICHH has been insightful in and of itself. But as Lynn Martin of the University of South Florida points out, in a practical sense, the ICHH has helped spur the emergence of ecological or "wild" immunology—the idea of studying immunology *in situ*, in wild animals, outside of the lab. The ICHH, he argues, has also contributed to the field of psychoneuroimmunology. This field "embraces the intuitive but not-in-a-textbook mindset that the immune system and the endocrine system and the nervous system are one intertwined thing." Over time, says Martin, there has been a huge increase in interactions between the biomedical world and the eco-evolutionary biologists. That is something he and others would like to see increase. So although a seemingly simple relationship between testosterone and immune function continues to be elusive, it is a hypothesis that still bears fruit. By pushing, merging, and expanding the boundaries of ecology, animal behavior, endocrinology, and immunology, its offshoots have the potential to solve puzzles in human and veterinary medicine and perhaps even extend the boundaries of how we understand gender.

Rebecca Adrian has studied sexual signaling in canaries, whose plumage colors are influenced by carotenoid pigments in their diet. In her experimental studies, she found that carotenoids do not affect immune function. Photograph: Rebecca Adrian.

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