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Abstracts

ENDOCRINE-IMMUNE INTERACTIONS IN MALE *SCELOPORUS* LIZARDS: TESTING AN ASSUMPTION OF THE IMMUNOCOMPETENCE HANDICAP HYPOTHESIS

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Variation in male secondary sexual traits contributes to explaining variation in reproductive success. Regulation of secondary sex traits is often mediated by the endocrine system, especially testosterone. Although high testosterone can enhance sexual characters and competitive ability, it may also prejudice immune function, a key assumption of the "immunocompetence handicap hypothesis". Little work in reptiles has tested these assumptions. We experimentally assessed the testosterone immunosuppression assumption in adult male *Sceloporus undulatus* lizards captured during the breeding season. We manipulated circulating androgen levels and then measured two aspects of immune function that reflect humoral and cellular responses, respectively. We surgically castrated males and/or implanted them with testosterone capsules to alter plasma androgen levels. Following primary and secondary immunizations with a standard immunogen (phthalate-conjugated Keyhole Limpet Hemocyanin, pt-KLH), we measured humoral antibody responses with a competitive ELISA. We compared this to humoral responses produced using the sheep RBC hemagglutination protocol, to assess the efficacy of each protocol. To estimate cell-mediated immune responses, we used a delayed-type hypersensitivity test involving subcutaneous injections of antigen (pt-KLH) versus saline and measuring differences in swelling 24-hrs post-injection. Swelling primarily reflects T-cell migration into the injection site. Using radioimmunoassay, we measured plasma levels of testosterone and corticosterone to validate the hormone manipulations and to assess any immunosuppressive effects of high corticosterone, which could increase due to immune challenges and/or surgery treatments.

Keywords: *Testosterone, immunocompetence, lizards, pt-KLH*

EFFECTS OF TESTOSTERONE AND IMMUNE CHALLENGE ON WHITE BLOOD CELL COUNTS IN MALE *SCELOPORUS UNDULATUS* LIZARDS

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Testosterone affects behavior and morphological traits important in sexual selection. Testosterone also plays a role in the development and functioning of immune system components. The immunocompetence handicap hypothesis (Folstad and Karter, 1992) proposes that tradeoffs, mediated by plasma levels of testosterone, exist between the immune system and the elaboration of secondary sexual traits. Endocrine-immune interactions are well documented in many species, and steroid hormones can be immunoenhancing or immunosuppressive.

Relatively little work has examined this in reptiles. Using testosterone implants and/or castration, we asked if circulating levels of testosterone cause changes in immune parameters in adult male *Sceloporus undulatus* lizards. We assessed how an antigenic challenge (phthalate-conjugated Keyhole Limpet Hemocyanin) affects these measures in males with and without altered testosterone. We calculated the average proportion of each WBC type, total WBC counts, hematocrit, and heterophil to lymphocyte ratio (H:L ratio). The ratio of these two most abundant circulating leukocytes is used in many studies as a diagnostic indicator of stress (Gross, 1981). We found that total WBC counts, the proportion of heterophils, and the H:L ratio differed among treatment groups after testosterone manipulation and immune challenge.

Keywords:

SIZE MATTERS, BUT BIGGER IS NOT ALWAYS BETTER: FEMALES, PREDATORS AND GENITAL EVOLUTION

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Male genitalia may experience more rapid, divergent evolution than any other animal character—but why? Research during the past several decades has culminated in the view that genital diversification primarily results from postmating sexual selection (e.g. sperm competition, cryptic female choice). However, the potential roles of premating sexual selection (e.g. mate choice) and natural selection have received little attention. We examined the possible importance of these mechanisms by investigating divergence in male genitalia between populations differing in predator regime for two species of livebearing fish (*Gambusia affinis* in Texas, USA and *G. hubbsi* in the Bahamas). Controlling for body size, males exhibited a larger gonopodium (sperm-transfer organ) in predator-free environments than in predatory environments—a trend that persisted across space (multiple populations), time (multiple years), and species. By conducting laboratory experiments with *G. affinis*, we found that premating sexual selection appears to favor larger male genitalia (females exhibited mating preference for males having larger gonopodia), but natural selection in the presence of predatory fishes seems to favor reduced genital size (larger gonopodium size was associated with reduced burst-swimming performance, an important antipredator behavior). These findings contrast with the prevailing views regarding genital evolution, and suggest a need to reconsider the current paradigm for genital diversification.

Keywords: *phenotypic differentiation, natural selection, predation, sexual selection*

ALL THE RIGHT MOVES: PHYLOGENETICALLY WIDESPREAD NATRICINE PROMISCUITY

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Promiscuity and the occurrence of multiple paternity within litters of offspring are characteristic of many species within the animal kingdom, and are analogous to the mating strategies of cross-pollinating plants. Specific knowledge of reptile mating systems, however, is lacking. Previous independent investigations determined multiple paternity to be characteristic of four disjunct

natricine snake species, including *Thamnophis sirtalis*, *Nerodia sipedon*, *Thamnopis butleri*, and *Thamnophis elegans*. We investigated the various untested clades and subclades of North American natricine snakes to determine the phylogenetic extent of multiple paternity within this subfamily. Representative species tested included members of the semifossorial clade (*Storeria dekayi*, *Storeria occipitomaculata*), watersnake clade (*Nerodia rhombifer*, *Regina septemvittata*) and gartersnake clade (*Thamnophis radix* – widespread subclade; *Thamnophis sauritus* – ribbon and common gartersnake subclade; *Thamnophis melanogaster* – Mexican subclade). Using primers originally developed for *Thamnophis* and *Nerodia*, DNA from litters with known mothers was amplified at highly variable microsatellite loci. After identifying maternal alleles in offspring genotypes, the number of paternal alleles per litter was counted. The presence of more than two paternal alleles (the maximum when a sire is heterozygous) within any single litter indicated that multiple paternity is widespread throughout Natricinae, with the exception of the Mexican subclade, represented by *T. melanogaster*. This study is the first to examine multiple paternity across a widespread, related group of vertebrates.

Keywords: *multiple paternity*, *snake*, *natricine*, *microsatellite DNA*.