

## The Evolution of Sexually Selected Traits and Antagonistic Androgen Expression in Actinopterygian Fishes

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**ABSTRACT:** Many sexually selected traits in male fishes are controlled by testosterone. Directional selection for male ornaments could theoretically increase male testosterone levels over evolutionary time-scales, and when genetically correlated, female testosterone levels as well. Because of the negative fitness consequences of high testosterone, it is plausible that female choice for sexually selected traits in males results in decreased female reproductive fitness. I used comparative analysis to examine the association between male peak testosterone expression and sexually selected ornaments. I also tested for genetic correlation between male and female androgen levels. The presence of sexually selected traits in males was significantly correlated with increased peak androgen levels in males as well as females, and female testosterone levels were significantly correlated with male peak testosterone titers, although the slope was only marginally <1. This suggests that selection to decouple high male and female testosterone levels is either weak or otherwise ineffective.

**Keywords:** testosterone, sexual antagonism, genetic correlation, supertree, comparative analysis.

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Numerous sexually dimorphic traits in the ray-finned (actinopterygian) fishes are the result of sexual selection (Reimchen 1989; Basolo 1990; Houde and Endler 1990; Stott and Poulin 1996; Harrington 1997; Marcus and McCune 1999; Kuwamura et al. 2000; Amundsen and Forgren 2001; Curtis and Stoddard 2003; Kortet et al. 2003, 2004). Several of these traits have been linked to testosterone and 11-ketotestosterone (11KT) expression or receptors, including sexual dichromatism (Asahina et al. 1991), elongate fins (Zauner et al. 2003), electric mating

calls (Bass and Hopkins 1983; Bass and Volman 1987; Landsman and Moller 1988; Freedman et al. 1989), and breeding tubercles (Kortet et al. 2003, 2004). A teleost-specific androgen, 11KT is a secondary derivative of testosterone and is primarily confined to males (Borg 1994).

Theoretically, if sexually selected traits are linked to testosterone or 11KT, directional selection for exaggerated traits would result in increased androgen expression in males, although this relationship may be ephemeral, as there is evidence that males evolve alternative mating strategies to avoid costly sexually selected traits (Mank and Avise 2006a). Because 11KT is converted from testosterone, directional selection for increased 11KT would likely require increased testosterone as well. Directional selection for increased androgen production in males might be linked to an initial associated testosterone increase in females when genetic correlation between female and male testosterone expression levels is strong (Roff 1997), as has been documented in some avian taxa (Ketterson et al. 2005; Møller et al. 2005).

Concomitant with its role in regulating male ornaments, testosterone is a known immunosuppressant in vertebrates (Duffy et al. 2000; Zysling et al. 2006), including fish (Folstad and Karter 1992; Slater et al. 1995; Skarstein and Folstad 1996; Kortet et al. 2003; Vainikka et al. 2005), although the relationship between immunosuppression and sexual selection in fish is complex and is still the subject of some debate (Hamilton and Zuk 1982; Wedekind 1992; Taskinen and Kortet 2002). The benefits of increased reproductive fitness in males (through increased mating success due to territoriality, mating behavior, or sexually selected ornaments) would be expected to outweigh the immunosuppressive effects of the elevated testosterone. If there were a correlated increase in female testosterone levels, females would presumably suffer immune suppression without benefits to reproductive fitness. This would result in a sexually antagonistic hormone expression profile, and selection would therefore act to decouple male and female testosterone levels.

The ray-finned (actinopterygian) fishes are an excellent clade for comparative analyses of the evolution of sexually selected traits, because there are numerous easily observed

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male ornaments that have been shown to be polyphyletic and highly labile in previous comparative appraisals of the Actinopterygii (Mank et al. 2005, 2006; Mank and Avise 2006a) when mapped onto the recently developed actinopterygian supertree phylogeny (Mank et al. 2005). The utility of this clade for comparative evolutionary evaluations of male ornaments (reviewed in Mank and Avise 2006b), in conjunction with the above-demonstrated links between sexually selected traits and testosterone, presents an opportunity to evaluate the degree of hormonal antagonism in the evolution of actinopterygian fishes.

There were two aims to this analysis. I first assessed the correlation between testosterone expression and sexually selected male ornaments. I then tested whether there was a correlated increase in female testosterone expression profiles in order to determine the degree of genetic correlation between male and female androgen expression. In the latter analysis, I examined the slope of the regression between male and female peak testosterone levels in order to gain insight into the degree of selective forces acting against high female androgen levels.

#### Material and Methods

I collected 66 accounts from the current literature describing male and female plasma steroid hormone profiles for 58 species. I recorded peak natural testosterone levels for adults of both sexes, as well as the secondary derivative androgen 11KT (typically confined to males only), standardizing all reports to nanograms of hormone detected per milliliter of plasma. "Peak hormone level" refers to the natural high point of androgen expression, which can fluctuate over the course of the spawning cycle. This metric represents an average across individuals during the seasonal apex. When multiple accounts existed for the same species, I averaged peak hormone levels. In the few studies where hormone profiles had been reported for different types of male mating strategies, I used the profile for the bourgeois male tactic, as this is the strategy most subject to both female choice and male competition and therefore most closely linked to sexual selection. Bourgeois males express sexually selected traits and defend spawning territories, as opposed to sneaker and satellite males, which usually lack these secondary sexual attributes (Taborsky 2001). All data were then log transformed to achieve a normal distribution. The references used to build this database are available in the appendix in the online edition of the *American Naturalist*.

I also collected accounts of sexually selected traits from the literature. I attempted to avoid problems with reproducibility and ascertainment bias in assessing the presence of sexually selected traits by using only accounts from field guides, aquarium manuals, and species descriptions. As in

previous comparative appraisals of sexually selected traits in ray-finned fishes (Mank et al. 2005, 2006; Mank and Avise 2006a; Mank, forthcoming), I focused on four sexually dimorphic traits that have been shown unambiguously to be the result of sexual selection in fishes: dichromatism (Reimchen 1989; Houde and Endler 1990; Stott and Poulin 1996; Amundsen and Forgren 2001), elongate fins (Basolo 1990; Harrington 1997; Marcus and McCune 1999; Kuwamura et al. 2000), breeding tubercles (Kortet et al. 2003, 2004), and electric mating calls (Curtis and Stoddard 2003); these data are also available in the appendix. Some of these traits may be present to a lesser degree in females (such as with nuptial coloration in salmonids), although they are always noticeably more pronounced in males. This approach is conservative in that it avoids traits, such as size and structural dimorphisms, that may have resulted in some degree from natural selection. I also omitted behavioral data from these analyses because they are not available for a wide range of taxa and because the relationship between behavior and sexual selection is not well understood across the actinopterygian clade.

I performed both phylogenetically corrected and uncorrected analyses on the data. I first analyzed the data without correcting for shared ancestry, where each species is an independent data point regardless of its evolutionary relationships. I then regressed log-transformed male and female plasma testosterone levels and computed a correlation coefficient ( $r^2$ ) and slope of the best-fit line. I also computed correlation coefficients for male testosterone and 11KT, female testosterone and male 11KT, and finally total male androgens (testosterone plus 11KT) and female testosterone. I then compared the male peak plasma testosterone and 11KT levels and the female peak plasma testosterone levels for species with and without sexually selected traits using a Mann-Whitney  $U$ -test. This statistic, which tests for significantly different distributions in two samples, reduces the influence of outliers.

I used independent contrasts (Felsenstein 1985) to correct for shared ancestry, using the topology of the actinopterygian supertree (Mank et al. 2005). While the independent-contrast method can incorporate branch length information, the amalgamated nature of the supertree prevents divergence estimates; therefore, all branch lengths were coded as equal, as recommended by Purvis et al. (1994). I first used CAIC (comparative analysis by independent contrasts; Purvis and Rambaut 1995) to estimate the phylogenetically corrected correlation between the log-transformed male and female peak testosterone levels, male testosterone and 11KT, and female testosterone and male 11KT, forcing the regression through the origin (Purvis and Rambaut 1995). I then assessed the phylogenetically corrected relationship between increased plasma levels of tes-

tosterone and 11KT and the presence of sexually selected male ornaments. For this latter analysis, the nature of the data set (dichotomous presence or absence of sexually selected traits and continuously distributed hormone levels) violates many underlying assumptions of CAIC. I therefore used sister-taxa comparisons to compare the hormone profiles (female peak testosterone, male peak testosterone, and male peak 11KT) for each species in my data set that exhibited manifestations of sexual selection with the closest relative that lacked such traits. Sister taxa diverged at the same point and are therefore by definition equally old (Cra-craft 1981). This eliminates the need to correct for divergence time and nested phylogeny. The overall trend in these comparisons was assessed with a Wilcoxon sign test (Sokal and Rohlf 1995; Vamosi and Vamosi 2005).

### Results

I found recorded plasma steroid profiles in the current literature for 58 species. Average peak testosterone levels (ng/mL) were 28.5 (range 0.25–340) in males and 36.0 (range 0.01–420) in females. These levels are not significantly different from each other ( $t$ -test,  $P = .3$ ; Mann-Whitney  $U$ -test,  $P = .5$ ). The derivative androgen 11KT averaged 113.3 ng/mL (range 1–320) in males and was either undetectably low or absent in females.

For all the hormones assessed here (male testosterone, male 11KT, and female testosterone), the presence of sexually selected traits was correlated with increased steroid hormone expression, when compared to species that lacked sexually selected traits, as shown in table 1. This trend was evident both with and without phylogenetic correction for shared ancestry.

Correlations between peak androgen expression profiles are shown in figure 1. For all phylogenetically uncorrected assessments, correlations between peak androgen titers (male and female testosterone levels, male testosterone plus 11KT) were positive and significant ( $P < .001$  in all cases). The analyses in which shared ancestry was corrected via independent contrasts were less straightforward, although there was a general positive relationship between peak androgen titers. All relationships showed a positive

correlation and slope, although only the relationships between female and male peak testosterone ( $P < .001$ ) and between female testosterone and total male androgens ( $P < .04$ ) were significant. For both the phylogenetically independent and corrected analyses between female and male peak testosterone levels, the slope of the regression line was less than 1, though only slightly less in both cases (phylogenetically uncorrected slope = 0.91, SE = 0.014; phylogenetically corrected slope = 0.83, SE = 0.0013).

### Discussion

The correlation between sexually selected traits in ray-finned fishes and increased testosterone and 11KT in males (table 1) suggests that female choice and/or male-male competition based on male ornaments results in directional selection for increased androgen titers in males. Because 11KT is a derivative of testosterone (Borg 1994), it is likely that increased testosterone is a prerequisite for increased 11KT, as suggested by the positive relationship between male testosterone and 11KT (fig. 1B, 1F). Although the phylogenetically corrected relationship between testosterone and 11KT was not significant, this is likely due to the limited number of possible independent contrasts (27) compared to data points (58). The interconnection between testosterone and 11KT makes it difficult to untangle the relationship between sexually selected traits and specific androgens. I therefore cannot conclude from this data set whether sexually selected traits are directly correlated with increased testosterone or testosterone is only an intermediate between sexual selection and 11KT.

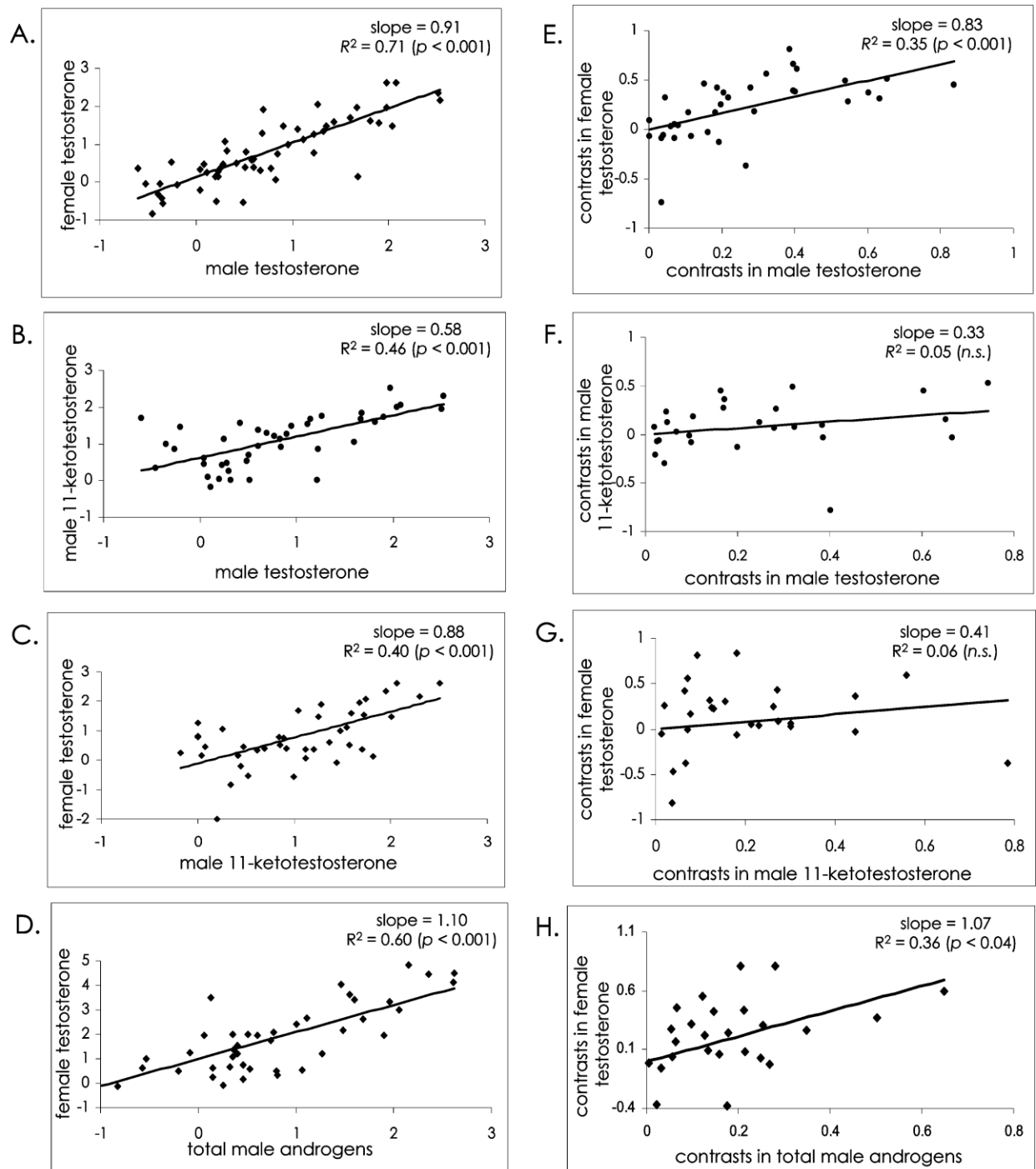
There is evidence of a correlated increase in females due to directional selection for male androgens, although the data suggest that female testosterone is more correlated with male testosterone than with male 11KT. First, on average, the peak female testosterone titer is significantly higher for species in which males exhibit sexually selected ornaments than for sexually monomorphic species (table 1). Additionally, female testosterone is significantly correlated with male testosterone in both the uncorrected and corrected analyses (fig. 1A, 1E). Both these lines of evidence offer indirect support for directional selection on increased tes-

**Table 1:** Comparison of peak androgen profiles for actinopterygian species with and without sexually selected traits

	Mean (ng/mL) for species without sexual selection (mean rank)	Mean (ng/mL) for species with sexual selection (mean rank)	Significance ( $P$ ) <sup>a</sup>	Significance ( $P$ ) <sup>b</sup>
Male testosterone	28.34 (26.7)	29.04 (37.7)	.02	.006
Male 11-ketotestosterone	24.67 (19.7)	73.12 (30.8)	.009	.03
Female testosterone	18.71 (26.2)	106.95 (37.7)	.01	.004

<sup>a</sup> As assessed by the Mann-Whitney  $U$ -test. Profile not corrected for shared ancestry.

<sup>b</sup> As assessed by the Wilcoxon sign test. Profile corrected for shared ancestry.



**Figure 1:** Correlations between peak androgen expression levels. *A, E*, Peak male and female testosterone. *B, F*, Peak male 11-ketotestosterone and testosterone. *C, G*, Peak male 11-ketotestosterone and female testosterone. *D, H*, Total peak male androgen (testosterone + 11-ketotestosterone) and female testosterone. All data are in nanograms per milliliter and are log transformed. *A–D* are not corrected for phylogeny; *E–G* have been corrected for shared ancestry via independent contrasts.

tosterone levels in males producing correlated increased testosterone in females. The evidence for correlated evolution between male 11KT and female testosterone is not as clear (fig. 1C, 1G). While the phylogenetically uncorrected analysis shows a clear and significant positive correlation, the phylogenetically corrected analysis is positive, though non-significant. The lack of significance in the analysis via independent contrasts is also likely due to the reduced number of possible independent contrasts (27) compared to species data points (58). Not surprisingly, there is further evidence for a positive coevolution between total female testosterone and total male androgens (fig. 1D, 1H).

The slope of the best-fit regression between female and male testosterone is significantly, though only marginally less than 1 in both the phylogenetically controlled and uncontrolled analyses. These slopes are far greater than those found in birds (Møller et al. 2005), where it is thought that females suffer costs with no increased reproductive fitness due to increased testosterone levels (Clotfelter et al. 2004; Zysling et al. 2006). This suggests that although there has been some force acting to decouple male and female testosterone expression levels in fish, the force is either weak or ineffective. This is somewhat surprising, given the documented immunosuppressive effects of testosterone in fishes (Folstad and Karter 1992; Slater et al. 1995; Skarstein and Folstad 1996; Kortet et al. 2003; Vainikka et al. 2005), which would, in theory, negatively affect females in species experiencing correlated responses to directional selection.

There are several possibilities that could explain this result. Because sexually selected traits can evolve quickly in fishes (Endler 1980; Basolo 1990; Meyer 1997; Mank et al. 2006), it is entirely possible that there has not been sufficient time for selection to decouple male and female testosterone titers. However, numerous studies have shown that fishes are remarkably sensitive to evolutionary pressures and can quickly respond to selection by evolving complex physiological, behavioral, and genomic adaptations (Reznick et al. 2002; Mank et al. 2005, 2006; Volff 2005; Mank and Avise 2006c). Additionally, many fish species exhibit multiple male mating strategies (Taborsky 2001). Males that exhibit cheating mating strategies often lack sexually selected traits and express lower androgen titers than dominant males (Brantley et al. 1993; Carneiro et al. 2003). If males with alternative mating strategies exhibit reduced androgen levels, why do females not do so as well? Therefore, it seems unlikely that selection against high female testosterone levels would not produce stronger results relatively quickly.

An alternative hypothesis is that females gain some sort of benefit from increased testosterone titers. In some species, testosterone induces expression of sexually selected male ornaments in females, though to a lesser degree than in males. Red coloration exhibited by salmonids is a good

example, in which females exhibit bright coloration, though much less than males (Froese and Pauly 2004). Possible benefits could come from reverse sexual selection, where males would select females based on some sexually selected trait, such as dichromatism. This type of scenario has been documented in fishes (Amundsen and Forgren 2001), although it seems likely only in situations of sex role reversal (Jones and Avise 1997; Jones et al. 2001), which are relatively rare and therefore cannot account for the overarching trend seen here.

Another possible benefit to females may lie in the indeterminate growth pattern of most fishes, in which body size increases throughout the life of a fish, though at different rates depending on various factors (Helfman et al. 1997). Testosterone is one of these factors, through its derivative of estradiol, and has been positively correlated with increased growth rates in fishes (Fagerlund et al. 1979; Ufodike and Oki 1991; Onuma et al. 2004). Increased body size in females could be advantageous in some circumstances and could therefore represent a beneficial effect of increased testosterone.

In many species, females are larger than conspecific males (Breder and Rosen 1966), probably because of the requisite energetic burdens in producing massive amounts of roe. In general, larger females produce more eggs (Kjesbu et al. 1998; Trippel and Neil 2004; Melo and Le Clus 2005). This suggests that there may be an indirect reproductive payoff to females for elevated testosterone (and therefore estradiol) expression: elevated testosterone increases body size, which in turn increases fecundity via roe production. There is also evidence that female body size may be a sexually selected trait, because males of some species select larger females for spawning partners when given a choice (Beeching and Hopp 1999; Dosen and Montgomerie 2004). The benefits of increased body size to roe production and mate selection may, at least in some cases, counter the immunosuppressive effects of elevated testosterone in females.

This line of reasoning can be extended further. Because this type of comparative analysis cannot determine directionality, it is entirely possible that selection for increased female body size and roe production is the cause of correlated increased male testosterone. However, this directionality of the coevolution of male and female testosterone is not supported by the finding that the presence of sexually selected traits in males is correlated with increased testosterone. Selection acting on females to increase body size cannot explain the evolution, exaggeration, and persistence of sexually selected traits in males, because these are energetically costly ornaments that often lead to increased predation (Endler 1980).

Finally, it is feasible that testosterone is not sexually antagonistic in fishes. If females lack androgen receptors,

then they would not be as sensitive to genetically correlated increased androgens resulting from directional selection on males. While it is unlikely that females lack androgen receptors entirely, it is possible that they lack receptors in some tissues and therefore have a partially insulated phenotype. If females possess such insulated phenotypes, there would be fewer negative consequences of increased testosterone levels for females, and therefore selection to decouple male and female testosterone levels would be reduced. This scenario is doubtful, however, since in many dimorphic species, females exhibit reduced expression of male sexually selected traits. Again, the most common example of this is in the salmonids, where females often show reduced levels of male nuptial coloration (Froese and Pauly 2004). If females had insulated phenotypes because of an absence of androgen receptors, it seems illogical that they would exhibit androgen-mediated coloration. Given the fact that females therefore likely possess androgen receptors and the documented negative effects of high androgens in male fishes (Slater et al. 1995; Skarstein and Folstad 1996; Kortet et al. 2003; Vainikka et al. 2005), it seems unlikely that there are no negative consequences in females of correlated increases in androgen expression.

It is important to point out that comparative phylogenetic analyses, though powerful for detecting general trends across deep evolutionary time, suffer from the inherent flaw of their strictly correlative nature. Without further manipulative experiments, it is not possible to conclusively identify causal relationships with comparative methods. This is the case in this analysis, because all the results presented here are purely correlative.

The clear positive association between sexual selection and androgen expression in this analysis is surprising, given the complexity of reproduction in ray-finned fishes. There are a multitude of mating strategies (Taborsky 2001), parental care strategies (Blumer 1979), and social systems (Oliver 1997) in this clade, and all these factors have been shown to intertwine with sexual selection (Leonard 1993; Mank et al. 2005; Mank and Avise 2006a). This suggests that the association between sexually selected traits and increased androgens in males, as well as a correlated increase in conspecific females demonstrated in this analysis, is a strong general evolutionary trend in this clade, although further empirical evidence will be needed to support this notion.

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#### Literature Cited

- Amundsen, T., and E. Forgren. 2001. Male mate choice selects for female coloration in a fish. *Proceedings of the National Academy of Sciences of the USA* 98:13155–13160.
- Asahina, K., T. Kobashi, and H. Soeda. 1991. Changes in plasma electrolyte concentrations and hormone concentrations during homing migration of chum salmon *Oncorhynchus keta*, with special reference to the development of nuptial color. *Nippon Suisan Gakkaishi* 57:599–605.
- Basolo, A. L. 1990. Female preference predates the evolution of the sword in swordtail fish. *Science* 250:808–810.
- Bass, A. H., and C. D. Hopkins. 1983. Hormonal control of sexual differentiation: changes in electric organ discharge waveform. *Science* 220:971–974.
- Bass, A. H., and S. F. Volman. 1987. From behavior to membranes: testosterone-induced changes in action potential duration in electric organs. *Proceedings of the National Academy of Sciences of the USA* 84:9295–9298.
- Beeching, S. C., and A. B. Hopp. 1999. Male mate preference and size-assortative pairing in the convict cichlid. *Journal of Fish Biology* 55:1001–1008.
- Blumer, L. S. 1979. Male parental care in ray-finned fishes. *Quarterly Review of Biology* 54:149–161.
- Borg, B. 1994. Androgens in teleost fishes. *Comparative Biochemistry and Physiology C* 109:219–245.
- Brantley, R. K., J. C. Wingfield, and A. H. Bass. 1993. Sex steroid levels in *Porichthys notatus*, a fish with alternative reproductive tactics, and a review of the hormonal basis for male dimorphisms among teleost fishes. *Hormones and Behavior* 27:332–347.
- Breder, C. M., and D. E. Rosen. 1966. *Modes of reproduction in fishes*. Natural History Press, Garden City, NY.
- Carneiro, L. A., R. F. Oliveira, A. V. M. Canário, and M. S. Grober. 2003. The effect of arginine vasotocin on courtship behaviour in a blennioid fish with alternative reproductive tactics. *Fish Physiology and Biochemistry* 28:241–243.
- Clotfelter, E. D., D. M. O'Neal, J. M. Gaudio, J. M. Casto, I. M. Parker-Renge, E. A. Snajd, D. L. Duffy, V. Nolan, and E. D. Ketterson. 2004. Consequences of elevating plasma testosterone in females of a socially monogamous songbird: evidence of constraints on male evolution? *Hormones and Behavior* 46:171–178.
- Cracraft, J. 1981. Pattern and process in paleobiology: the role of cladistic analysis in systematic paleontology. *Paleobiology* 7:456–468.
- Curtis, C. C., and R. K. Stoddard. 2003. Mate preference in female electric fish, *Brachyhyppopomus pinnicaudatus*. *Animal Behaviour* 66:329–336.
- Dosen, L. D., and R. Montgomerie. 2004. Female size influences mate preferences of male guppies. *Ethology* 110:245–255.
- Duffy, D. L., G. E. Bentley, D. L. Drazen, and G. F. Ball. 2000. Effects of testosterone on cell-mediated and humoral immunity in non-breeding adult male European starlings. *Behavioral Ecology* 11:654–662.
- Endler, J. A. 1980. Natural selection on color patterns of *Poecilia reticulata*. *Evolution* 34:76–91.
- Fagerlund, U. H. M., J. R. McBride, and E. T. Stone. 1979. A test of 17 $\alpha$ -methyltestosterone as a growth promoter in a coho salmon

- hatchery. *Transactions of the American Fisheries Society* 108:467–472.
- Felsenstein, J. 1985. Phylogenies and the comparative method. *American Naturalist* 125:1–15.
- Folstad, I., and A. J. Karter. 1992. Parasites, bright males, and the immunocompetence handicap. *American Naturalist* 139:603–622.
- Freedman, E. G., J. Olyarchuk, M. A. Marchaterre, and A. H. Bass. 1989. A temporal analysis of testosterone-induced changes in electric organs and electric organ discharges of mormyrid fishes. *Journal of Neurobiology* 20:619–634.
- Froese, R., and D. Pauly, eds. 2004. FishBase. <http://www.fishbase.org>.
- Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: a role for parasites? *Science* 218:384–387.
- Harrington, M. E. 1997. Behavior patterns and sexual dimorphism in the spotted dragonet *Diplogrammus pauciradiatus* (Pisces: Calionymidae). *Bulletin of Marine Science* 60:872–893.
- Helfman, G. S., B. B. Collette, and D. E. Facey. 1997. *The diversity of fishes*. Blackwell Science, Malden.
- Houde, A. E., and J. A. Endler. 1990. Correlated evolution of female mating preferences and male colour patterns in the guppy *Poecilia reticulata*. *Science* 248:1405–1407.
- Jones, A. G., and J. C. Avise. 1997. Polygynandry in the dusky pipefish *Syngnathus floridae* revealed by microsatellite DNA markers. *Evolution* 51:1611–1622.
- Jones, A. G., D. Walker, and J. C. Avise. 2001. Genetic evidence for extreme polyandry and extraordinary sex-role reversal in a pipefish. *Proceedings of the Royal Society B: Biological Sciences* 268:2531–2535.
- Ketterson, E. D., V. Nolan, and M. Sandell. 2005. Testosterone in females: mediator of adaptive traits, constraint on sexual dimorphism, or both? *American Naturalist* 166(suppl.):S85–S98.
- Kjesbu, O. S., P. R. Witthames, P. Solemdal, and W. M. Greer. 1998. Temporal variations in the fecundity of Arcto-Norwegian cod (*Gadus morhua*) in response to natural changes in food and temperature. *Journal of Sea Research* 40:303–321.
- Kortet, R., J. Taskinen, A. Vainikka, and H. Ylonen. 2003. Breeding tubercles, papillomatosis, and dominance behavior of male roach (*Rutilus rutilus*). *Ethology* 110:591–601.
- Kortet, R., J. Vainikka, M. J. Rantala, I. Jokinen, and J. Taskinen. 2004. Sexual ornamentation, androgens, and papillomatosis in male roach (*Rutilus rutilus*). *Evolutionary Ecology Research* 5:411–419.
- Kuwamura, T., K. Karino, and Y. Nakashima. 2000. Male morphological characteristics and mating success in a protogynous coral reef fish. *Journal of Ethology* 18:17–23.
- Landsman, R. E., and P. Moller. 1988. Testosterone changes the electric organ discharge and external morphology of the mormyrid fish, *Gnathonemus petersii* (Mormyriiformes). *Experientia* 44:900–903.
- Leonard, J. L. 1993. Sexual conflict in simultaneous hermaphrodites: evidence from serranid fishes. *Environmental Biology of Fishes* 36:135–148.
- Mank, J. E. Forthcoming. Mating preferences, sexual selection and patterns of cladogenesis in ray-finned fishes. *Journal of Evolutionary Biology*. doi: 10.1111/j.1420-9101.2006.01251.x.
- Mank, J. E., and J. C. Avise. 2006a. Comparative phylogenetic analysis of male alternative reproductive tactics in ray-finned fishes. *Evolution* 60:1311–1316.
- . 2006b. The evolution of reproductive and genomic diversity in ray-finned fishes: insights from phylogeny and comparative analysis. *Journal of Fish Biology* 69:1–27.
- . 2006c. Supertree analysis of the role of viviparity and habitat in the evolution of atherinomorph fishes. *Journal of Evolutionary Biology* 19:734–740.
- Mank, J. E., D. E. L. Promislow, and J. C. Avise. 2005. Phylogenetic perspectives on the evolution of parental care in ray-finned fishes. *Evolution* 59:1570–1578.
- Mank, J. E., D. W. Hall, M. Kirkpatrick, and J. C. Avise. 2006. Sex chromosomes and male ornaments: a comparative evaluation in ray-finned fishes. *Proceedings of the Royal Society B: Biological Sciences* 273:233–236.
- Marcus, J. M., and A. R. McCune. 1999. Ontogeny and phylogeny in the northern swordtail clade of *Xiphophorus*. *Systematic Biology* 48:491–522.
- Melo, Y. C., and F. Le Clus. 2005. Growth and reproduction of the pelagic goby *Sufflogobius bibarbatatus* off the Orange River, southern Africa. *African Journal of Marine Science* 27:265–273.
- Meyer, A. 1997. The evolution of sexually selected traits in male swordtail fishes (*Xiphophorus*: Poeciliidae). *Heredity* 79:329–337.
- Møller, A. P., L. Z. Garamszegi, D. Gil, S. Hurtrez-Bousses, and M. Eens. 2005. Correlated evolution of male and female testosterone profiles in birds and its consequences. *Behavioral Ecology and Sociobiology* 58:534–544.
- Oliver, A. S. 1997. Size and density dependent mating tactics in the simultaneously hermaphroditic seabass *Serranus subligarius* (Cope 1870). *Behaviour* 134:563–594.
- Onuma, T., H. Ando, N. Koide, H. Okada, and A. Urano. 2004. Effects of salmon GnRH and sex steroid hormones on expression of genes encoding growth hormone/prolactin/somatolactin family hormones and a pituitary-specific transcription factor in masu salmon pituitary cells in vitro. *General and Comparative Endocrinology* 143:129–141.
- Purvis, A., and A. Rambaut. 1995. Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analyzing comparative data. *Computer Applied Biosciences* 11:247–251.
- Purvis, A., L. Gittleman, and H.-K. Luh. 1994. Truth or consequences: effects of phylogenetic accuracy on two comparative methods. *Journal of Theoretical Biology* 167:293–300.
- Reimchen, T. E. 1989. Loss of nuptial color in threespine sticklebacks (*Gasterosteus aculeatus*). *Evolution* 43:450–460.
- Reznick, D. N., M. Mateos, and M. S. Springer. 2002. Independent origins and rapid evolution of the placenta in the fish genus *Poeciliopsis*. *Science* 298:1018–1020.
- Roff, D. 1997. *Evolutionary quantitative genetics*. Chapman & Hall, New York.
- Skarstein, F., and I. Folstad. 1996. Sexual dichromatism and the immunocompetence handicap: an observational approach using Arctic charr. *Oikos* 76:359–367.
- Slater, C. H., M. S. Fitzpatrick, and C. B. Schrek. 1995. Androgens and immunocompetence in salmonids: specific binding of and reduced immunocompetence of salmonid lymphocytes exposed to natural and synthetic androgens. *Aquaculture* 136:363–370.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry: the principle and practice of statistics in biology research*. W. H. Freeman, New York.
- Stott, M. K., and R. Poulin. 1996. Parasites and parental care in male upland bullies (Eleotridae). *Journal of Fish Biology* 48:283–291.
- Taborsky, M. 2001. The evolution of bourgeois, parasitic, and cooperative behaviors in fish. *Journal of Heredity* 92:100–110.

- Taskinen, J., and R. Kortet. 2002. Dead and alive parasites: sexual ornaments signal resistance in the male fish, *Rutilus rutilus*. *Evolutionary Ecology Research* 4:919–929.
- Trippel, E. A., and S. R. E. Neil. 2004. Maternal and seasonal differences in egg sizes and spawning activity of northwest Atlantic haddock (*Melanogrammus aeglefinus*). *Canadian Journal of Fisheries and Aquatic Science* 61:2097–2110.
- Ufodike, E. B. C., and O. O. Oki. 1991. Effect of testosterone proportionate on somatic and gonad growth in African catfish (*Clarias lazera*). *Journal of Aquatic Sciences* 6:31–34.
- Vainikka, A., E. I. Jokinen, R. Kortet, S. Paukku, J. Pirhonen, M. J. Rantala, and J. Taskinen. 2005. Effects of testosterone and  $\beta$ -glucan functions in tench. *Journal of Fish Biology* 66:348–361.
- Vamosi, S. M., and J. C. Vamosi. 2005. Endless tests: guidelines for analysing non-nested sister-group comparisons. *Evolutionary Ecology Research* 7:567–579.
- Volff, J.-N. 2005. Genome evolution and biodiversity in teleost fish. *Heredity* 94:280–294.
- Wedekind, C. 1992. Detailed information about parasites revealed by sexual ornamentation. *Proceedings of the Royal Society B: Biological Sciences* 247:169–174.
- Zauner, H., G. Begemann, M. Mari-Beffa, and A. Meyer. 2003. Differential regulation of *msx* genes in the development of the gonopodium, an intromittent organ, and of the “sword,” a sexually selected trait of swordtail fishes (*Xiphophorus*). *Evolution and Development* 5:466–477.
- Zysling, D. A., T. J. Greives, C. W. Breuner, J. M. Casto, G. E. Demas, and E. D. Ketterson. 2006. Behavioral and physiological responses to experimentally elevated testosterone in female dark-eyed juncos (*Junco hyemalis carolinensis*). *Hormones and Behavior* 50:200–207.

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