

NEWS AND VIEWS

Perspective

ELLEN D. KETTERSON

*Department of Biology, Indiana University, Bloomington, IN 47405, USA***Abstract**

This issue of *Molecular Ecology* includes an exciting article by Aubin-Horth *et al.* in which they examine behaviour, hormone levels, and gene expression in dominant and subordinate male and female cichlid fishes of the African species *Neolamprologus pulcher*. Their fascinating experiments take us one important step closer to an understanding of one of life's persistent mysteries: why males differ from females and how such differences develop and evolve.

Because males and females share much the same genome, the developmental emergence of typical sex differences requires mechanisms that promote sex-limited expression of genes. In those cases in which females assume masculine traits, an important question arises: Do females develop male traits through cross-sexual transfer whereby they tap into molecular and hormonal mechanisms that evolved in males (West-Eberhard 2003), or do they produce a convergent phenotype that arises through different developmental and activational pathways (e.g. Davis & Marler 2003; Rubenstein & Wikelski 2005)? West-Eberhard (2003, p. 262) established six criteria for recognizing cross-sexual transfer of masculine traits to females, and her list included derived female resemblance of males, maladaptive consequences of the transferred trait for females, and evidence that masculinization has a hormonal basis.

In vertebrates, sex-limited expression often involves androgens. These steroid hormones are named for their role in generating male morphology and behaviour, and one of them, testosterone, is commonly regarded as the 'male hormone'. Despite the common view, a spate of recent studies has focused on testosterone's role in females. Comparative studies of testosterone in birds, for example, have shown that plasma levels in females are higher in colonial than in solitary breeding species (Moller *et al.* 2005), and higher in monogamous birds than in birds with nonmonogamous mating systems (Ketterson *et al.* 2005). Both these findings suggest that testosterone may play an important adaptive role in mediating female behaviour. Other interspecific comparisons in birds and fish, in particular ones reporting positive covariation across species in plasma

levels of male and female testosterone, are more consistent with a nonadaptive role for testosterone in females (Ketterson *et al.* 2005; Moller *et al.* 2005; Mank 2007). Such covariation could be explained by directional sexual selection on male testosterone that has led to correlated responses in females. If such responses produced detrimental phenotypic effects in females, further male evolution might be constrained, at least temporarily (Mank 2007).

Recently, investigators have manipulated testosterone in female vertebrates to determine whether experimental elevation of their testosterone would induce expression of male-like traits, and whether this expression would benefit or harm the females. For example, in female dark-eyed juncos (*Junco hyemalis*, a songbird), experimentally elevated testosterone enhances aggression and suppresses immune function, just as it does in male juncos (Zysling *et al.* 2006). On the other hand, parental behaviour of female juncos is unaffected – is not suppressed – by experimental elevation of testosterone (Clotfelter *et al.* 2004), even though testosterone does suppress parental behaviour in males of this and other bird and fish species (Oliveira 2004). Thus, the manipulative studies suggest that traits typically mediated by testosterone in males can in some – but not all – cases be similarly mediated in females; these traits might be expected to spread in females if selection were to favour them.

To take the study of cross-sexual transfer of behaviour to another level, much more needs to be known about the downstream impact of testosterone on the phenotype of females exhibiting male-typical traits, including interactions with other hormones and the particular genes expressed. An ideal system would be one in which some females naturally express a male-typical trait and other females do not. The existence of the trait would be *prima facie* evidence that it had evolved despite any costs it might impose on

Correspondence: Ellen D. Ketterson, Fax: 812-855-6705; E-mail: ketterso@indiana.edu



Fig. 1 The cichlid fish species *Neolamprologus pulcher*, in which dominant behaviour in females is associated with male-like gene expression and testosterone levels. (Photo credit: Julie Desjardins)

females, and the variation in expression among females would indicate that the trait was inducible and could therefore provide insight as to patterns of gene expression. The question would be whether the patterns indicate cross-sexual transfer or convergence.

Aubin-Horth *et al.* report on just such a system in this issue of *Molecular Ecology*. They investigated territorial aggression in females of the cichlid fish *Neolamprologus pulcher*, a native of Lake Tanganyika. *N. pulcher* breeds cooperatively and forms social groups consisting of a breeding pair and a variable number of helpers. The breeders maintain their territories year-round, and the breeding female is at least as aggressive as her mate, sometimes more so. Helpers aid in rearing offspring and are socially subordinate to the dominant breeders. The existence of dominant and subordinate members of each sex allowed the researchers to investigate whether aggression and dominance are achieved by similar pathways in females and males, as would be predicted by cross-sexual transfer.

Aubin-Horth *et al.* observed social behaviour in 14 groups of *N. pulcher*, each group consisting of a male and female breeder and a male and female helper. They quantified aggressive behaviour and determined, as expected, that breeders were dominant to helpers. In fact, female breeders were the most aggressive class of fish. When Aubin-Horth *et al.* ascertained blood hormone levels they found that the dominant females had the highest levels of plasma testosterone.

The authors also extracted brain tissue and quantified the mRNAs present. To this end they employed a custom-made cichlid cDNA microarray developed for a related species. Eighteen fish representing the four nodes (breeder male, breeder female, helper male, helper female) were read on the microarray, all 18 were read two at a time to assess relative gene expression and also read individually.

Using stringent statistical criteria for inclusions, the authors identified four genes that were expressed differentially according to status (dominant vs. subordinate, regardless of sex). One candidate gene was expressed more strongly in dominant males and females; three other genes were expressed more strongly in subordinates. When transcribed, the gene associated with dominance is known to produce a small but powerful peptide hormone, arginine vasotocin or AVT, which has been associated experimentally and neuroanatomically with social behaviour in fish, amphibians, and birds (Goodson & Bass 2001). Arginine vasopressin (AVP), which differs from AVT by only a single amino acid, plays a similar important role in mammalian social behaviour. AVP has even been touted as the 'monogamy hormone' because it facilitates pair bonding in males of the monogamous prairie vole (*Microtus ochrogaster*), a species in which it also influences aggression towards strangers (Stribley & Carter 1999). Importantly, AVT is often modulated by testosterone and plays a greater role in male than in female aggression (Goodson & Bass 2001).

Having identified genes whose expression varied with dominance status, Aubin-Horth *et al.* (2007) then looked for covariation between hormone levels and gene expression. They found that when individuals were combined across sex and dominant/subordinate status, plasma testosterone covaried positively with AVT expression. In addition to testosterone, fish employ another androgen, 11 keto-testosterone (11-KT), and interestingly, 11-KT was higher in males than in females regardless of dominance status.

Finally, Aubin-Horth *et al.* looked for genes that differed in relative expression at any two of the four nodes, including sex and dominance comparisons, and they identified 36 genes whose expression varied by sex, status, or both. They used a statistical clustering program to classify the 18 individuals according to their overall expression profiles at these 36 genes. Six of the 18 fish were dominant breeders (three males and three females) and 12 were subordinates (six males, six females). In a fascinating result, they found that the fish formed two clusters. The first cluster consisted of all but one of the males plus the three dominant females, while the second cluster consisted of the remaining male, which was a subordinate, and the six subordinate females. That is, the known-to-be aggressive, dominant breeding females clustered with males, both dominant and subordinate, and, with one exception, the subordinate females formed their own cluster. Said still another way, the dominant females were not only masculinized in their behaviour, they appear to have accomplished this by employing male-like patterns of gene expression.

Future studies will have a number of important issues to pursue and sort out. First, in the present study, the four genes including AVT that were related to dominance and subordination might just as easily have been attributed to reproductive status, because the dominant fish were also

breeders and the subordinate fishes were all nonbreeding helpers. Thus, effects on gene expression induced by dominance status will need to be distinguished from those induced by reproducing or helping.

Similarly, the dominant breeders were members of a pair bond while the subordinate helpers were not, leaving open whether the elevated AVT expression might relate to their bonded status (compare prairie voles above), as well as or instead of their dominance status. AVT is known for the variety of roles it plays and for its flexibility as to whether it is suppressive or enhancing of affiliation or aggression (Goodson & Bass 2001). It will be exciting to learn from future studies where in the brain of *N. pulcher* the AVT gene is being expressed, where AVT's receptors are located, and what the receptors' role is in the induction of various kinds of social behaviour.

To conclude, Aubin-Horth *et al.* have enhanced our understanding of sex differences by focusing on a case in which some but not all females resemble males by participating in territorial aggression. By comparing hormones and gene expression in dominant and subordinate individuals of both sexes, they have identified genes whose expression covaries with social status. Their findings lead the way towards a deeper understanding of how individuals come to vary in their behaviour, as well as to a greater appreciation for how sex-typical and atypical behaviour might evolve in relation to social system.

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Dr. Ellen Ketterson studies animal and bird behavior, focussing on the effects of hormones on parental care, dominance, aggression, mating effort, and other aspects of bird behavior and phenotype. Her research has led to a greater understanding of how selection acts on many hormone-mediated traits, in a wide variety of avian species.
