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Perspective

The ART of brain expression

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When trying to find a mate, one might think about using a dating app. Imagine that someone else has installed the same app and tries to access the same potential mates that you have chosen, and that this someone uses false facts about himself/herself to increase the chance of dating someone before you or anyone else with honest status information does. Sounds familiar? It actually is, and in no way is such comportment restricted to human courtship behaviour. Alternative reproductive tactics (ARTs) are widespread in the animal kingdom. In this issue of Molecular Ecology, Cardoso, Gonçalves, Goesmann, Canário, and Oliveira (2017) investigate plastic ARTs of the peacock blenny (*Salaria pavo*), in which males occur in three morphs: nestholders, sneakers and transitionals between the two former. They apply transcriptome sequencing to answer the question how brain gene expression contributes to sex role-specific behaviour and to intersex phenotypes.

KEYWORDS
behavior/social evolution, evolution of sex, fish, phenotypic plasticity, transcriptomics

From a molecular perspective, much progress in identifying the differences between females and males has been made through the study of sex chromosomes and—probably even more so—the analysis of differential gene expression patterns (for a review see, e.g., Grath & Parsch, 2016). The examination of species with reversed sex roles or with alternative sex morphs adds a novel view to this question, allowing to decouple gene expression patterns related to reproductive functions from those related to reproductive behaviour. Investigating such particular species helps us understand the relative contribution of differential gene expression to behavioural variation and, ultimately, to phenotypic plasticity.

The peacock blennies investigated by Cardoso et al. (see Figure 1) feature a sex-role reversal, with females courting and breeding with several males whereas solely nestholder males provide parental care to broods and invest in female attraction, secondary sexual characters (SSCs) and breeding territories (Gonçalves, Almada, Oliveira, & Santos, 1996). In addition to these territorial males, there are sneaker males that mimic females phenotypically and behaviourally in order to fertilize eggs deposited in the nestholders' territories. Depending on their birth date, sneakers change into nestholders in their first (transient pathway, only short phase of being a sneaker) or second breeding season (Fagundes et al., 2015).

In their study, Cardoso et al. established a de novo transcriptome to then assess expression in brain tissue from females, territorial and sneaker males. Importantly, they also included "transitional males," which do not anymore show female mimicking but also not yet nestholder SSCs. This latter group is reproductively inactive, probably due to focusing their energy investment into somatic growth. The experimental design including territorial, sneaker and transitional males allowed to disentangle sex-specific expression potentially implicated with reproduction from sex role-specific behaviour as well as sex role-specific phenotypes.

Overall, they found that all four phenotypes separated according to their brain transcriptomes—not only differentiating sexes but clearly, and even more so, behavioural morphs, implying that neurogenomic states correspond to behavioural statuses at reproduction. Characterizing the morph-specific gene expression patterns, it further appeared that downregulation of gene expression is more specific within each phenotype than upregulation.

Another important observation is that of all four phenotypes, sneaker males showed the most divergent gene expression patterns. This could be a more general pattern, which has been observed in several other species featuring ARTs (e.g., Partridge, MacManes, Knapp, & Neff, 2016; Stiver, Harris, Townsend, Hofmann, & Alonzo,

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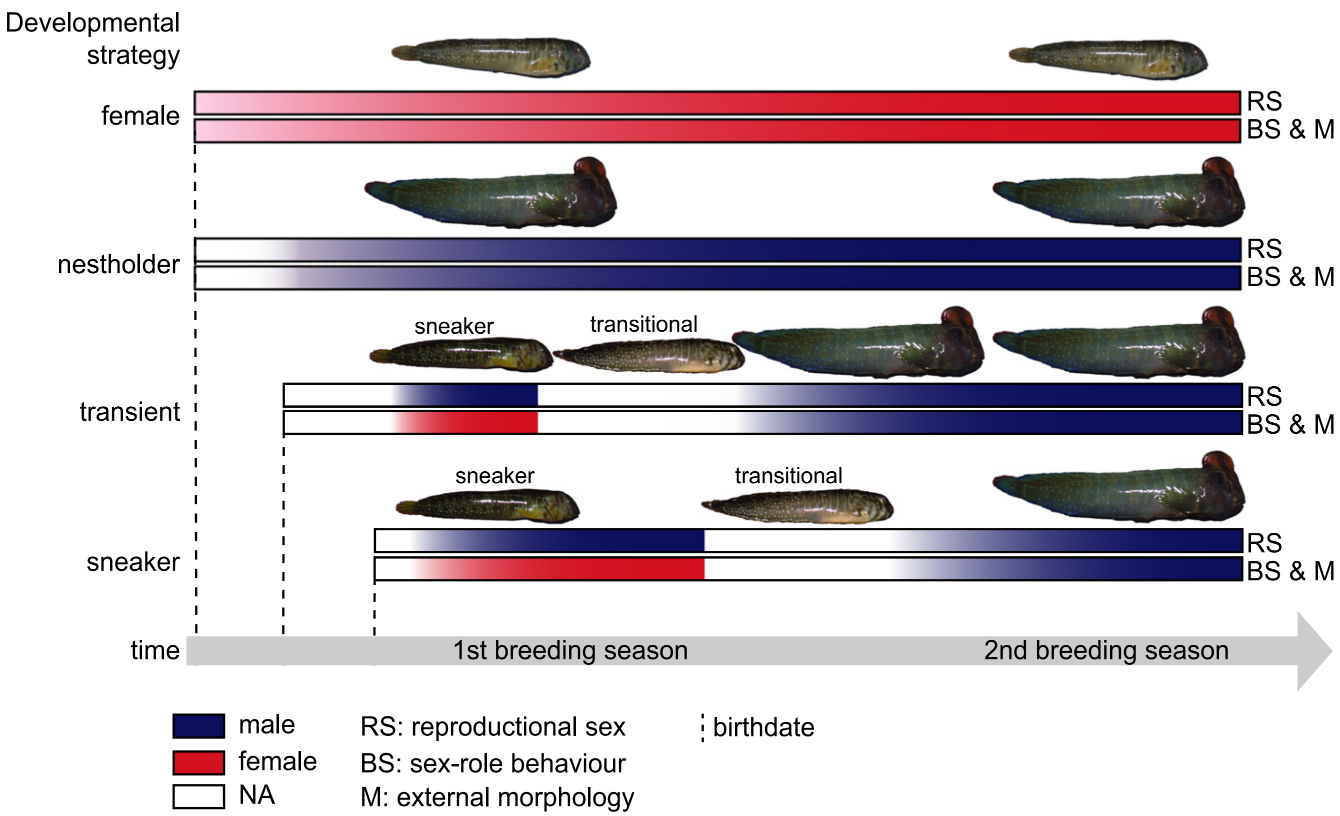

FIGURE 1 Males of the peacock blenny (*Salaria pavo*) population investigated by Cardoso et al. (2017) can follow three different developmental strategies. Depending on their birth date, they either reproduce directly as territorial nestholder males or they reproduce shortly as parasitic sneaker males and change within their first breeding season to the nestholder morphology (transient strategy) or they reproduce their first season as sneakers and only in their second season as nestholders (sneaker strategy). Females of this population are the courting sex. Sneaker males mimic female behaviour as well as body coloration to get access to fertilization of eggs laid in nestholder territories. Transitional males lose the female traits and stop to reproduce until they become nestholders. Fish images courtesy of Sara D. Cardoso and Rui F. Oliveira

2015), however not a ubiquitous one (Stuglik, Babik, Prokop, & Radwan, 2014). Interestingly, in the peacock blenny, the global brain expression profile of females is most similar to the one of territorial males, followed by transitional males and the very distinct sneakers. This suggests that overall brain expression profiles reflect behaviour rather than sex. Sneakers clearly resemble an intersex state, having the male sex and reproduction but mimicking female behaviour and nuptial coloration. This probably requires a complex neurogenomic regulation, leading to their divergent gene expression profile. Transitional males, on the other hand, appear to be at the edge of leaving this state, showing a pattern of more downregulation of genes and overall the lowest level of overexpression.

In a second step of the analysis, Cardoso et al. focused on sex-biased transcripts (defined as differentially expressed between nestholders and females). These clearly show an intersex pattern in sneaker males, with male-biased genes showing lower expression levels in sneakers compared to nestholders, and female-biased genes showing higher expression levels in sneakers compared to nestholders. Transitional males showed the same pattern as sneakers. For these two subsets of genes, the clustering patterns were different compared to the overall pattern. When considering only nestholder-biased genes, all male morphs clustered together, whereas sneakers and transitionals clustered with females for female-biased genes. The neurogenomic signature thus corresponded to the behavioural intersex status. Within the male morphs, for the nestholder-biased genes, transitional males form a cluster with the nestholders, suggesting a higher degree of demasculinization of the sneaker gene expression compared to the transitionals. Or it could indicate that gene expression in transitional males is approaching the one in nestholder males, as transitionals will eventually turn into nestholders. (Recall that only nestholders express male-specific reproductive behaviour and male SSCs.)

Conducting a gene expression network analysis, Cardoso et al. further showed that nestholder males are associated with a gene expression module involved in energy production likely indicative of the metabolic requirements of reproduction. Nestholder males usually do not leave nests and hence have little access to new energy intake. The network analysis also revealed a downregulation of G-protein-coupled-receptor (GPCR) pathway in sneakers, which might suggest that their brain is less susceptible to (steroidal) gonad signalling via these receptors.

Gene-ontology (GO) analysis of female-biased genes that also show upregulation in sneakers but not in transitionals suggests a role for histone modification as well as regulation of Ral (ras-like) protein signal transduction in female courtship behaviour. Likewise, GO analysis of genes exclusively overexpressed in nestholder and sneaker males suggests that cell cycle regulation might play a role in active male reproduction. A total of 19 transcripts were solely upregulated in nestholder males making them candidates for the control of male reproductive behaviour which is not expressed in sneakers or transitional males.

The demasculinization of sneaker and transitional males might follow a similar trajectory in gene expression and reflects their intersex status. But what sets them apart and explains the female behaviour in sneakers? There remains a small set of female-biased transcripts that is overexpressed in sneakers but not in transitional males with a potential function in neural plasticity that could represent a shared genetic repertoire involved in female courtship behaviour.

On a gene-by-gene level, Cardoso et al.'s data provide little support for the existence of major conserved expression of certain genes across ART species. Nevertheless, aiming at the big picture, they point out that enzymes responsible for post-translational modifications of histones and for repression of DNA transcription could play a general role in ARTs. Epigenetic impacts on the expression of behaviour receive more and more interest. Their study revealed that *dnmt3a*, an enzyme responsible for de novo methylation, is upregulated in females, sneakers and transitional males, hinting towards a role for epigenetic regulation in female behaviour.

Clearly, however, more data are needed to understand the action of epigenetic modifications as well as GPCR signalling on steroidal action and coupled behaviour. Still, the new data by Cardoso et al. fit convincingly to previous observations in the same species that suggest that neuronal levels of estradiol are lower in sneakers as in nestholder males, which could be coupled to brain feminization. This study links earlier work on steroid status and its impact on behaviour with the transcriptomic response and regulation of it.

Depending on the birth date, the life history of a peacock blenny male can involve three different stages of "maleness" that are wired and rewired through the regulation of gene expression in the brain and coupled to steroid hormone status. Beyond the detailed exploration of one ART system, this paper can help us understand which genes play how and when a role in reproductive processes. Cardoso et al. show that condition-dependent reproductive plasticity can be regulated through changing the levels of sex-biased genes. Gene expression needed to manifest the behaviour can dominate the neurogenomic state of an individual on top of its baseline required to maintain its sexual identity. So in the end, it might be gene expression that regulates how we date, no matter if one uses an app to find a mate or the nest of someone else.

AUTHOR CONTRIBUTIONS

A.B. wrote the manuscript.

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