

Mom and dad are not that different after all: immune modulation as a prerequisite for the evolution of pregnancy

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Abstract

Pregnancy, the post-fertilization period when embryos are incubated within the body, is a dynamic multistage process that has convergently evolved in many vertebrates. To increase independence from environmental fluctuations and protect offspring from predation, challenges had to be initially overcome. The most obvious, when considering such an intimate relation between the parent and its semi-allogenic offspring, was the pressing need to dodge immunity-associated embryo rejection. In mammals, immunological tolerance was found to be dependent on the active modulation of the immune system. Even though supporting much of the current knowledge on vertebrate pregnancy, mammals lack extant transitional stages that could help reconstruct the evolutionary pathway of this fascinatingly complex reproduction mode. In this issue of *Molecular Ecology*, Parker et al. (2022) selected an untraditional model - the seahorse and pipefish family, whose species evolved male pregnancy across an almost continuous gradient of complexity, from external oviparity to internal gestation. By contrasting gene expression profiles of syngnathids with distinct brooding architectures, this study allowed for the observation of subtle evolutionary adaptations, while confirming the existence of remarkable similarities to 'female' pregnancy (e.g., the evolution of male pregnancy in pouched species occurred alongside immune downregulation, and inflammation seems vital during early pregnancy stages). In a world where the debate on sex-roles takes centre stage, Parker et al. (2022) appealing results hint at the fact that the strongly convergent evolution of vertebrate pregnancy was seemingly unaffected by which sex carries the burden of gestation.

Mom and dad are not that different after all: immune modulation as a prerequisite for the evolution of pregnancy

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Pregnancy, the post-fertilization period when embryos are incubated within the body, is a dynamic multistage process that has convergently evolved in many vertebrates. To increase independence from environmental fluctuations and protect offspring from predation, challenges had to be initially overcome. The most obvious, when considering such an intimate relation between the parent and its semi-allogenic offspring, was the pressing need to dodge immunity-associated embryo rejection. In mammals, immunological tolerance was found to be dependent on the active modulation of the immune system. Even though supporting much of the current knowledge on vertebrate pregnancy, mammals lack extant transitional stages that could help reconstruct the evolutionary pathway of this fascinatingly complex reproduction mode. In this issue of *Molecular Ecology*, Parker et al. (2022) selected an untraditional model - the

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Syngnathids (seahorses, pipefishes, pipehorses and seadragons) belong to a diverse family of bony fish with more than 300 species, primarily recognized by its unique mode of reproduction: male pregnancy. This novelty of the syngnathid lineage long attracted attention from the scientific community and was rapidly recognised as significant to the

evolutionary radiation of the syngnathid lineage. In fact, one of the first attempts at reconstructing the phylogenetic relationships within syngnathids, long before the advent of genomics, was partially grounded on the general architecture of brooding structures (Herald, 1959). Curiously, recent efforts, backed by current methodologies (Hamilton et al., 2017; Stiller et al., 2022), still recognise the diversification of structures involved in male pregnancy as relevant for diagnosing syngnathid taxa. From an ancestral pipefish, that probably presented a rather simple brooding structure where eggs were exposed, increasingly more complex structures evolved up to the sealed seahorse brood pouch (Figure 1). This apparent gradient of complexity, easily observable in extant syngnathid species (and almost absent in mammals), renewed the interest in this family of quirky fish into as an invaluable opportunity to shed additional light on the evolution of pregnancy.



Figure 1: Two pregnant syngnathids, a pipefish (*Syngnathus abaster*) and a seahorse (*Hippocampus guttulatus*); photo by Patrick Louisy).

Even though viviparity has evolved more than 150 times in vertebrates (Whittington, Griffith, Qi, Thompson, & Wilson, 2015), most of our knowledge on the necessary immune system adjustments derives from the mammals' female pregnancy. Here, immunological tolerance to semi-allogenic embryos is achieved by the downregulation of both major histocompatibility complex pathways (MHC I and II). So, what evolutionary solutions emerged with syngnathid male pregnancy? Recently, Roth et al. (2020) showed, by reconstructing the immune gene repertoire of syngnathid genomes, that the evolution of syngnathid pregnancy coincided with adjustments to the adaptive immune system. In seahorses (genus *Hippocampus*) and pipefish (of the genus *Syngnathus*), both comprising species with a closed brood pouch (where the risk of embryo rejection would be theoretically higher), several genomic modifications of the MHC II pathway were uncovered, involving either loss or modifications of strategic genes. Even though the precise rearrangement of the MHC II pathway differed between the two types of studied brood pouches, the end result is similar and indicative of selection for lower immunological vigilance during the evolution of pregnancy. Moreover, by analysing the expression of immune and pregnancy genes in reproductive tissues, Roth et al. (2020) found considerable overlap between male and female pregnancy, suggestive of a process of convergent evolution.

The work of Parker et al. (2022), while seemingly flowing from the leading-edge findings of Small, Harlin-Cognato, and Jones (2013), Whittington et al. (2015) and Roth et al. (2020), introduced some relevant methodological upgrades. To fully understand a dynamic process such as pregnancy, where a myriad of cryptic mechanisms occur in orderly succession, one needs to go beyond a couple of snapshots. Thus, Parker et al. (2022) not only extended the inner brood surface tissue gene expression analyses to four species, thus further solidifying the validity of their results, but they also contrasted gene expression at different pregnancy stages (nonpregnant, early, late and parturition), in three distinct brooding architectures (external egg brooding surface, inverted brood pouch, and sealed brood pouch; Figure 2).

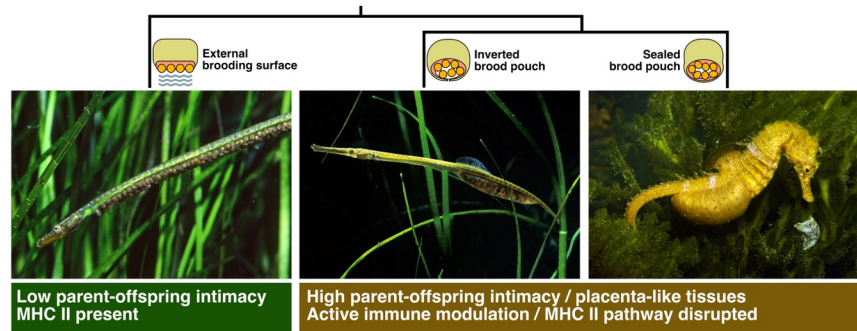


Figure 2: The three types of brooding structures investigated by Parker et al. (2022), and the simplified corresponding syngnathid phylogeny (from left to right: *Nerophis ophidion*, *Syngnathus typhle* and *Hippocampus guttulatus*). Photos by Patrick Louisy).

By sequentially monitoring male pregnancy, Parker et al. (2022) were able to highlight, with great detail, striking similarities between male and female pregnancy, ranging from: i) the existence of clear stage-specific gene expression patterns, denoting the necessary morphological and physiological changes that occur in the pregnant parent, ii) the need for an inflammatory process during early pregnancy, likely assisting egg engulfment, or iii) the presence of homologous gene expression profiles. The apparent absence of male pregnancy specific genes is particularly interesting, reinforcing Whittington et al. (2015) observation that pregnancy regulation, despite independently evolving in clearly divergent evolutionary lineages, seems to use a common 'genes toolkit'.

By contrasting male pregnancy along syngnathids' gradient of brooding structure complexity, Parker et al. (2022) reinforces the perception that attaining the highest degrees of intimacy between parent and offspring (i.e., gestation in a brood pouch) was only possible with immune modulation. As also observed by Roth et

al. (2020), the MHC I downregulation seems to actively contribute to the necessary immunological tolerance that circumvents rejection of developing offspring. While the exact role of MHC II remains ambiguous, we can be tempted to speculate that its simultaneous loss in pouched syngnathids is probably much more than a coincidence. Was MHC II dismantlement a by-product or a prerequisite to the transition from non-pouched to pouched pregnancy? As pipefishes and seahorses differ in the type of genomic rearrangements leading to MHC II impairment (Roth et al., 2020), I presently lean towards the second hypothesis. The future inclusion of additional syngnathid species, from the pool of intermediate pregnancy types positioned between external egg gluing and closed pouches, will surely help shed light on this topic. As an added bonus, a closer look into the most basal branches of syngnathid phylogeny will also help pinpoint the unidentified origin, and mode, of nutrient transfer to (and from) developing embryos. While evidence suggests that pouched species are able to exchange nutrients to and from their brood (Cunha, Berglund, Mendes, & Monteiro, 2018; Sagebakken, Ahnesjö, Mobley, Braga Goncalves, & Kvarnemo, 2009), the limited data on pouchless species (Miranda, Vieira, & Monteiro, 2017; Parker et al., 2022) leads to contradictory conclusions.

Once viewed as mere oddities, the extraordinary life histories of syngnathids have long converted them into model organisms to address a wide array of questions related to the evolution of reproductive complexity... and there are still secrets to uncover. Even if unable to decisively unveil all the cryptic mechanisms occurring during male pregnancy, Parker et al. (2022) work significantly progresses our understanding of the subcellular subtleties resulting from the evolution of an intimate, and apparently sex-undiscriminating, connection between offspring and the pregnant mother or father.

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