Evolutionary developmental biology

Early Palaeozoic dentine and patterned scales in the embryonic catshark tail

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1. INTRODUCTION
Recent work has highlighted the presence of scale patterning early in the development of certain chondrichthyan taxa, including both major chondrichthyan groups, the Holocephali and Elasmobranchii (e.g. Miyake et al. 1999; Didier 2004; Freitas & Cohn 2004; Eames et al. 2007). This patterning is observed in bilateral scale rows along the body and rows associated with the lateral sensory canals; patterned scale rows have also been described in association with the tail fin (axial lobe). These latter scales occur in four rows, two on each side of the tail, one dorsal and one ventral. Within each row, scales are equidistant from one another (Scyliorhinus canicula: Ballard et al. 1993; Cephaloscyllium ventriosum: Eames et al. 2007; Heterodontus portusjacksoni, Heterodonius galeatus: Johanson et al. 2007). All these scale rows are lost during ontogeny and replaced by more randomly developing scattered body scales. The body scales show nearest neighbour irregular spacing related to proximity of nearby scales providing initiation factors (M. Smith & R. Fraser 2007, personal observation) and are not organized into rows of iterative, evenly spaced scales (also Reif 1985).

Johanson et al. (2007) suggested that linearly patterned rows of scales were plesiomorphic for chondrichthians, as scale rows and a more strictly regulated scale development also characterized actinopterygians and sarcopterygians (Osteichthyes; Donoghue 2002; Sire & Akimenko 2004). The scattered chondrichthyan body scales were considered to be a derived feature within the clade. Differences in scale patterning between early, ordered rows and later developing, more scattered scales suggest that chondrichthyan scolation may be separated into discrete developmental modules (one for ordered rows and one for scattered scales). These separate developmental modules have different regulation and potentially different evolutionary histories.

Alongside this regular scale patterning, we observed that dentine in S. canicula (small-spotted catshark) tail fin scales is a type that is otherwise only known from the oldest shark scales, including those from the Ordovician Harding Sandstone (ca 450 Myr ago; Sansom et al. 1996) and those assigned to Elegestolepis from the Upper Silurian (ca 430 Myr ago; Karatajute-Talimia 1973). This structural similarity suggests that patterned tail scales have an extremely deep phylogenetic history, retained for 450 Myr but now restricted to a brief period of ontogeny in living sharks.

2. MATERIAL AND METHODS
Scyliorhinus canicula eggs from Tenby Aquarium (Pembrokeshire), Milport Field Station Station (Glasgow University) and the London Aquarium were held under standard aquarium conditions. Staged embryos (Ballard et al. 1993) were removed from egg cases, anaesthetized (MS222) and tails anterior to the caudal fin were removed. These were processed in 4% PFA for 4 days at 4°C, cryosectioned (10μm) and hybridized with a probe for Scshh (S. canicula sonic hedgehog gene), SEM or histological examination. Comparative material of fossil chondrichthyan scales was obtained from Harding Sandstone deposits, Colorado, USA (see also electronic supplementary material).

3. RESULTS
(a) Macroscopic appearance
Scanning electron micrographs, photomicrographs and photomicrographs show two dorsal and ventral, laterally opposed rows of scales spaced symmetrically and iteratively from the extreme tip of the tail of a S. canicula embryo (stage 33). The older scales are more caudal, with a more irregular shape (figures 1c, f, g (versus figure 1b, e) and 2a, b), but all show a flat smooth surface (figure 1). Scales develop within a distinct epithelial pocket (figure 2a) with individual positions dorsoventrally staggered by half a scale pocket (figure 2a, b, arrows in figure 2a, b pass between two dorsal scales but through one ventral scale). Consequently, the dorsal and ventral rows are

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non-aligned with one more scale in the dorsal row than the ventral (9/8 in stage 31, with 10/9 in stage 33), indicating a regulated sequential timing of individual scale row development, synchronized between the rows (figure 2a,b).

The wide, regular spacing within the row and the staggered temporal development are associated with a timing difference in focal expression of Scshh in the epithelium, each expression site localized to the scale pocket. This appearance with different levels of expression (figure 2c,d) confirms that the staggered timing of regulation is iterative in a caudal to rostral direction and also dorsal in advance of ventral (figure 2c,d). One new rostral scale, relative to older caudal scales (figure 2c), is characterized by a small rounded area of Scshh expression. This is a scale pocket, where all epithelium expresses Scshh compared with the older scales, where this expression becomes shifted to the posterior (caudal) margin of the scale (figure 2c, arrow).

**4. DISCUSSION**

Body scales (including those on the head) of *S. canicula* develop in an irregular, dispersed, scattered pattern, with the position of new scales probably controlled by their nearest neighbours (Reif 1985, pl. 26; M. Smith & R. Fraser 2007, personal observation). This non-regular, non-iterative arrangement is characteristic of chondrichthyan scale development (Reif 1985). *Scyliorhinus caniculus* lacks a single ‘initiator’ scale at the posterior extremity of the tail, differing from *Heterodontus* in this feature (Johanson et al. 2007), but otherwise the regular iterative pattern
is maintained. An iterative series implies regulated development of scale rows and data on Scshh expression illustrates one of the patterning genes used in this process, comparable to Shh patterning observed in zebrafish scales (Danio rerio; Sire & Akimenko 2004) and for sequential tooth pattern in the dentition of the trout (Onchorhyncus mykiss; Fraser et al. 2004). This regularity is plesiomorphic for chondrichthyans (phylogeny in the electronic supplementary information) and is retained only during early ontogenetic stages of living taxa (Johanson et al. 2007).

Dentine of the S. canicula tail scales is not orthodentine (regular cell process tubules formed from a layer of cells lining a central pulp cavity), but a branching pattern where wide canals branch into smaller and finer tubules. This is comparable to an ancient form of dentine lacking a central pulp cavity present in Ordovician shark scales (figure 2k,l; Sansom et al. 1996) and in those assigned to the Silurian Elagostolepis grossi (Karatajute-Talimaa 1973). Growth stages of the S. canicula tail scales show dentine development similar to the growth series...
described by Karatajute-Talimaa (1973, refigured in Smith & Hall 1990, fig. 1). These ancient scales have multiple dentine canals, housing putative odontoblast processes, but all placoid scales, as those of S. canicula body scales, have typical orthodentine (Reif 1980). Thus, S. canicula tail scale dentine is similar to that of the oldest fossil chondrichthyan rather than a modern type as in typical placoid scales.

_Elegestolepis_ and the Harding Sandstone taxa have not yet been resolved within a chondrichthyan phylogeny (Sansom et al. 1996), but they are generally believed to be phylogenetically basal forms. The tail scales of _S. canicula_ appear only during egg capsule development and not only retain a plesiomorphic organization or patterning (relative to osteichthians), but also an early, phylogenetically basal form of dentine that can be traced back over more than 400 Myr. More scattered body scales develop later in chondrichthyan ontogeny, composed of typical orthodentine. These observations indicate that chondrichthyan scales on different regions of the body can be considered as independent developmental modules. We suggest that the ‘patterned tail scale’ module represents the more ancient, relative to the scattered body scales, which may be related to suggestions that the tail is itself an independent developmental unit relative to the rest of the body (reviewed in Handrigan 2003). Data on pattern regulation and histology of tail scales in other taxa, and the histology of bilaterally axially patterned scales on the body during early ontogeny, for example, in holocephalans (Didier 2004) and other elasmobranches (Miyake et al. 1999; Freitas & Cohn 2004) would test these hypotheses.


