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"Research Note"

EMBRYONIC SURFACE CILIATED CELLS IN *ELEUTHERODACTYLUS URICHI* (ANURA: LEPTODACTYLIDAE)*

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Abstract – While our knowledge of anuran embryonic and larval surface ciliation and their diversity of ciliation patterns are significant, very few references were noticed on embryonic ciliation in direct-developing anurans, such as species in the genus Eleutherodactylus. *Eleutherodactylus urichi* embryos were found to have surface ciliated cells during much of their development until a few days before hatching. Ciliation was most prominent on the pharyngeal region and on the tail fins, both regions believed to have a respiratory role, and supporting the hypothesis that embryonic ciliation in amphibians serves principally to aid respiration by moving intracapsular fluid. The limb-buds were also well ciliated, an evolutionary novelty since in most anurans with a tadpole stage, surface ciliation regresses before limbs show significant development.

Keywords – *Eleutherodactylus*, direct development, embryonic ciliation

1. INTRODUCTION

Amongst the vertebrates, significant embryonic/larval surface ciliation has been reported only for lungfish [1, 2] and amphibians [3], though occasional isolated cilia are seen transiently in other kinds of embryos such as the dogfish [4] and the chick [5]. While carrying out a survey of anuran embryonic and larval surface ciliation [6], the scarcity of references to embryonic ciliation in direct-developing anurans, such as species in the genus *Eleutherodactylus* was noticed by the author.

Callery et al. [7] have pointed out that development in *Eleutherodactylus* involves a complex mix of features: some larval-specific structures such as adhesive glands, teeth, jaws and the lateral line have been deleted entirely; others such as the external gills, operculum and tail have been greatly reduced or highly modified.

In anurans with a normal larva, we have found [6] that surface ciliation is first seen in late neurula stages (Gosner stages 14, 15) [8] and persists through hatching (stages 17-20 for most species) until it regresses around the time that independent feeding begins (stages 25-26). Surface ciliation is not evenly distributed: some parts of the body are more heavily ciliated than others, and ciliation persists longer at some locations than others.

Several functions have been suggested for embryonic/larval ciliation [3]. Evidence is best for a respiratory role [9]: by moving the fluid within the confines of the vitelline membrane, surface ciliated cells promote gas exchange between the jelly layer and the embryo's surface. After hatching, this role may still be important for larvae hanging still in static waters. Another possible function is movement: surface cilia cause rotation of the embryo for a time, and allow newly hatched larvae to glide over the substratum

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[3]. However, it is not clear whether these movements are useful in some way, or merely a consequence of the mechanism for moving fluids.

In *Eleutherodactylus coqui*, Townsend & Stewart [10] reported rapid rotation at stage 3 (abbreviated hereafter as TS: TS3 is equivalent to Gosner stages 14-16), declining to slow or absent by stage 5 (no exact equivalent to Gosner stages, but a little after Gosner stage 18). Earlier, Gitlin [11] described embryonic rotation in *E. portoricensis* (regarded by Townsend & Stewart as *E. antillensis*): rotation possibly extended over a wider range of stages than reported by Townsend and Stewart, but was dependent on the degree of hydration of the eggs. Gitlin was puzzled by the mechanism of rotation, since he was unable to detect any cilia at the surface of the embryos. However, Adamson et al. [12] reported "numerous" columnar ciliated cells being visible in sections of the skin of *E. martinicensis* (actually *E. johnstonei*, according to Townsend and Stewart) at their stage III (equivalent to TS stage 5), but that these cells were absent by the next stage examined (stage 7). Adamson et al. [12] suggest that the function of the ciliated cells is to power embryo rotation.

Given this patchy previous evidence on surface ciliation, and the interest in how direct developing anurans have evolved from those with larvae [7], it seemed worthwhile to use scanning electron microscopy to map the distribution of ciliated cells on an eleutherodactylid embryo as an example of a larval feature in a direct-developing embryo.

2. MATERIALS AND METHODS

Surface ciliation in embryos of *Eleutherodactylus urichi* were investigated. *E. urichi* is believed to be a Trinidad and Tobago endemic [13] and is widely distributed in forested areas on both islands. Unfortunately, eggs are not easy to find. Over a period of years, only four clutches were found: one under a rotting log; one in an abandoned hummingbird nest, on the ground; and two on bromeliad leaves, close to the ground. Collections were made from May to July. The number of eggs in a clutch ranged from 6-12. As soon as eggs were collected, they were examined using a dissecting microscope to check their stage of development and health, then incubated on the surface of moist tissue, in a petri dish at 25°C. As the eggs developed, individual eggs were fixed in Bouin's fluid or in 2.5% glutaraldehyde in a phosphate buffer. Later, outer jelly capsules were peeled off and the embryos were staged according to Townsend & Stewart's system [10] for *Eleutherodactylus*. Some small differences between *E. urichi* and Townsend and Stewart's description of *E. coqui* were noticed, but there was no difficulty in assigning stages. *E. urichi* embryos seemed to be rather susceptible to fungal attack: one clutch was infected on collection and did not develop at all; in two others, development proceeded in the laboratory for a few days, and then stopped. The results are therefore based on a rather small number of healthy embryos. After staging, embryos were processed for scanning electron microscopy by standard methods, and then examined using a Phillips 500 scanning electron microscope. Images were examined over a range of magnifications from x24 to x3200 and recorded by Imageslave for Windows. In processing the embryos for SEM, removing much of the yolky tissue via the ventral side of each embryo prior to dehydration was helpful because previous experience showed that very yolky amphibian eggs tended to burst open at the critical point drying stage.

3. RESULTS AND DISCUSSION

E. urichi eggs near the start of incubation were 3.6±0.1 (n=10) mm in diameter and surrounded by a thick dense jelly coat. The eggs are not adhesive to one another. The precise incubation time of *E. urichi* is not known, but the most healthy batch was at the TS stage 4, two days after collection, and hatched 24 days later–giving a total incubation time of 26 days, or a little more, well in line with Townsend & Stewart's

17-26 days for *E. coqui*.

The distribution of ciliated cells on different parts of the body at the stages examined is shown in Table 1. The measurements are semi-quantitative in that the ratio of ciliated cells to non-ciliated cells were counted at three areas (low density=1:5; medium=1:10; high=1:20), then other areas were assessed and compared with these standards. The density of ciliated cells on any body region was not uniform and regions were graded into adjoining regions. Nevertheless, Table 1 gives a good indication of the pattern of ciliated cells and how it changed with each stage.

Table 1 Distribution of ciliated cells by body region and Townsend Stewart stage in *E. urichi*

	Stage								
Body region	4	5	6	7	8	9	11	12	14
Head - dorsal	0	0	0	*	*	*	0	0	0
Head - lateral	*	**	***	***	***	***	*	*	0
Trunk - dorsal	0	0	0	0	0	0	0	0	0
Trunk - lateral	*	*	*	*	*	*	*	*	0
Yolk-sac	*	n	*	*	*	*	*	*	n
Tail - stem	0	*	*	*	*	*	n	0	a
Tail - fins	0	0	**	**	**	**	n	0	a
Forelimbs	0	*	*	*	**	**	*	0	0
Hind limbs	0	*	*	*	*	*	*	0	0

0-no ciliated cells; *-low density; **- medium density;
***-high density;n-not available; a- structure absent

Figure 1 shows a selection of the body regions examined in *E. urichi* embryos and limb bud regions examined in some indirect developing anurans. Several features of these patterns are worth commenting on. Townsend & Stewart [10] estimated that *E. coqui* stage 4 was equivalent to Gosner stage 17-18, when many anuran embryos hatch: thereafter, there are no equivalents because of the extreme modifications of development in *Eleutherodactylus*. In anurans with tadpoles, it was found [6] that levels of ciliation peak at or just after hatching, then decline on most body regions until disappearing soon after the stage when feeding begins. In *E. urichi*, the time of the greatest abundance of ciliated cells was from TS stages 6-9, well after the equivalent period in anurans with conventional larvae. Ciliated cells in *E. urichi* did decline prior to hatching, but in eleutherodactylids, hatching is equivalent to metamorphosis in other anurans, as the time when the aquatic embryonic environment is exchanged for terrestrial conditions, and a terrestrial integument is required. It can be suggested that ciliated cell regression occurs in time to allow for juvenile cutaneous differentiation to be completed by hatching. It would be interesting to test whether ciliated cell regression is sensitive to thyroid hormone, as Callery & Elinson [14] have shown is the case for several late pre-hatching events in *E. coqui.*

The highest ciliated cell densities were found on the lateral side of the head, essentially the pharyngeal region, and on the tail 'fins'. In eleutherodactylid embryos, the tail fins are thin-walled highly vascularised and laterally expanded structures, thought to have a respiratory function. The occurrence of abundant ciliated cells on the pharyngeal region (external gills are reduced or absent in eleutherodactylids–[10]) and on the tail fins supports the hypothesis that ciliated cells are involved in assisting respiration. In species with tadpoles, it was found [6] that the tail is generally less heavily ciliated than several other areas. However, ciliated cells regressed some time prior to hatching, when the metabolic needs of the embryo are presumably highest. A possible explanation lies in Townsend & Stewart's observation [10] that from stage 9 onwards, the tail beings to thrash about, increasing strongly as development proceeds, with the limbs beginning to move. These observations suggest that internal fluid movements at later stages no longer require surface ciliated cells.

An unexpected finding was the occurrence of ciliated cells on the limb buds, with forelimbs having denser populations than hindlimbs. In anurans with tadpoles, the hind limb buds make their first appearance when surface ciliated cells have generally regressed, and forelimbs undergo their development covered by the operculum: neither have surface ciliated cells. Expansion of the ciliated cell area on to the limb buds is therefore a real evolutionary novelty in the eleutherodactylids, assuming that other species in the genus have similar patterns to *E. urichi*.

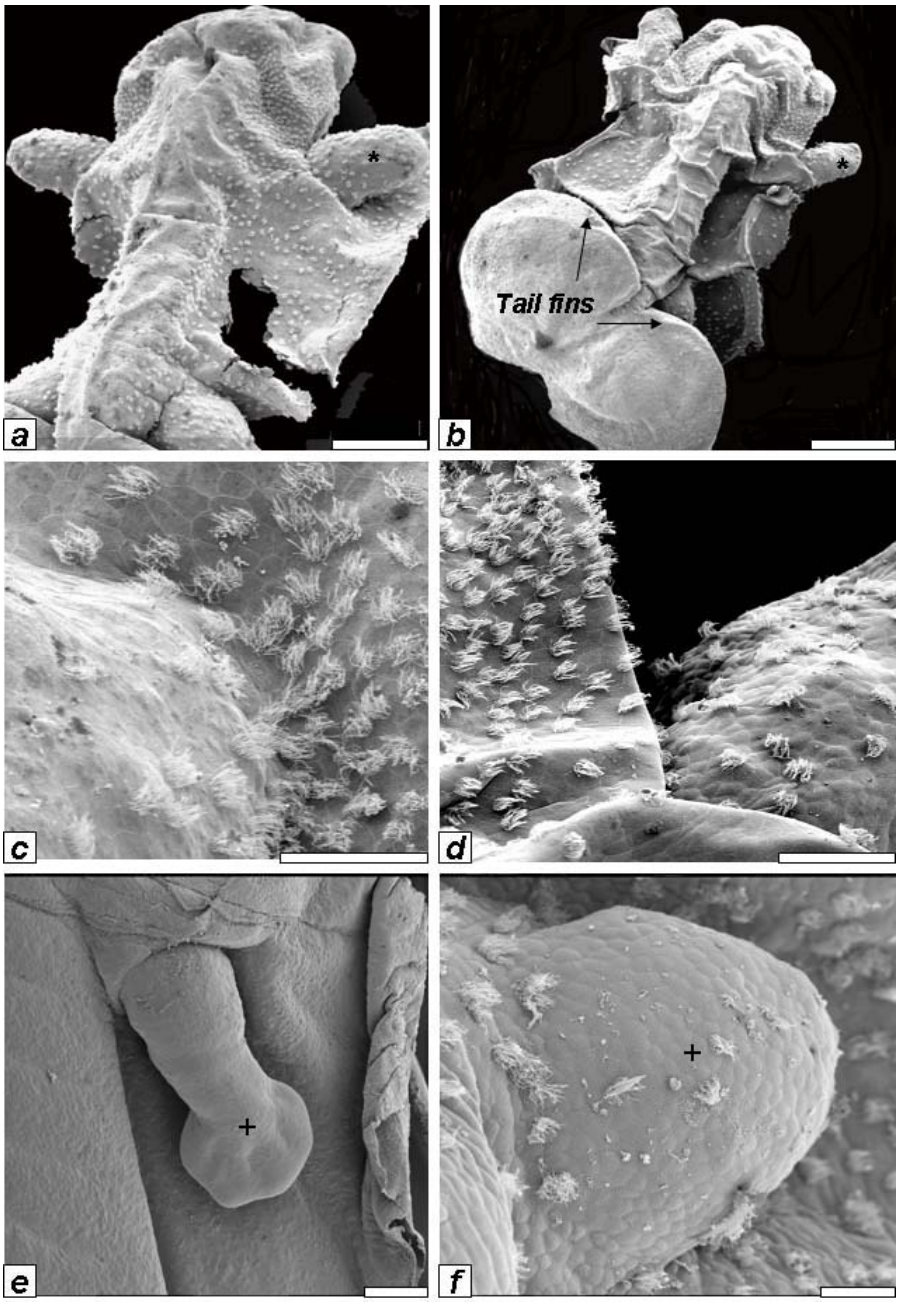

Fig. 1. Scanning electron micrographs to show distribution of ciliated cells on the surface of *E. urichi* at different stages and locations (a-d) and on the surface of hind limb bud for two other anuran species (e-f). a) stage 5, overall dorsal view, b) stage 6/7, overall dorsal view c) stage 5, dorso-lateral side of the head, d) stages 6/7, dorso-lateral side of the head and forelimb bud; e) *X. laevis*, Gosner stage 31, hind limb bud; f) *B. viridis*, Gosner stage 27, hind limb bud *=forelimb, +=hindlimb, Scale bars: a and e=500µm, b=1mm, c and d=150µm, f=60 µm

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