

## Cocoon Structure and Function in the Burrowing Hyloid Frog, *Pternohyla fodiens*

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**ABSTRACT**—The epidermal cocoon of *Pternohyla* consists of multiple sheddings of the stratum corneum interspersed with mucus filled subcorneal spaces. The rate of water loss through the cocoon is very low ( $0.6 \text{ mg g}^{-1} \text{ hr}^{-1}$ ) under the experimental conditions of flowing dry air. The cocoon of *Pternohyla* represents the first known case of cocoon formation in a hyloid frog.

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### INTRODUCTION

The presence of an epidermal cocoon in estivating amphibians was first reported by Lee and Mercer (1967) in some Australian leptodactylid frogs. Since then similar structures have been found in South American leptodactylids (McClanahan, et al., 1976), South African ranids (Loveridge, 1976; Loveridge and Craye, 1979) and the salamander *Siren intermedia* (Reno, et al., 1972). We here report cocoon formation in the North American hyloid, *Pternohyla fodiens*, and discuss some aspects of cocoon formation and function.

### MATERIALS AND METHODS

Six specimens of *P. fodiens* were collected near Sells, Pima Co., Arizona and kept in terraria with water available and fed crickets (*Achaeta*). Cocoon formation was induced by placing 2 frogs in individual containers with about 4 cm of water. The water was allowed to evaporate slowly and a cocoon was evident within four weeks. The time of initiation of cocoon formation was not determined since the frogs were left unobserved for many days.

Fragments of a cocoon were prepared for study with the electron microscope as in McClanahan, et al. (1976). Thick sections were stained with methylene blue and observed under the light microscope.

The evaporative water loss (EWL) of frogs in their cocoons was determined using the methods of Shoemaker, et al. (1972). Individual frogs were placed in a 875 ml leucite cylinder (9.4 cm × 12.6 cm) through which dry air was passed at 500 ml/min. The relative humidity and temperature of the outflowing air was measured with a HygroDynamics thermohygrometer. Surface area was calculated as in McClanahan and Baldwin (1969). The experiments were done at 23°C.

### RESULTS

The cocoon surrounding the *Pternohyla* is transparent and colorless. During cocoon formation the frog is motionless, with all limbs folded tightly against the body. At first glance the presence of

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the cocoon is not obvious—due to its close adherence to the body contours and its transparency. The cocoon is perforated at the nostrils but otherwise appears to cover the entire body surface. Though the frog is restrained by the cocoon and usually immobile it will respond to prodding by perceptible movements. The cocoon softens when placed in water and the frog initiates movements that liberate it from the cocoon.

Light microscopy of a cocoon fragment revealed a multilayered structure with a total thickness of 0.05 mm. A maximum of 43 cell layers were visible in the vertical section. Electron microscopy of the cocoon demonstrated the characteristic pattern of multiple squamous cell layers separated by less dense granular intercellular material (Fig. 1). Remnants of desmosomes, cell junctions between

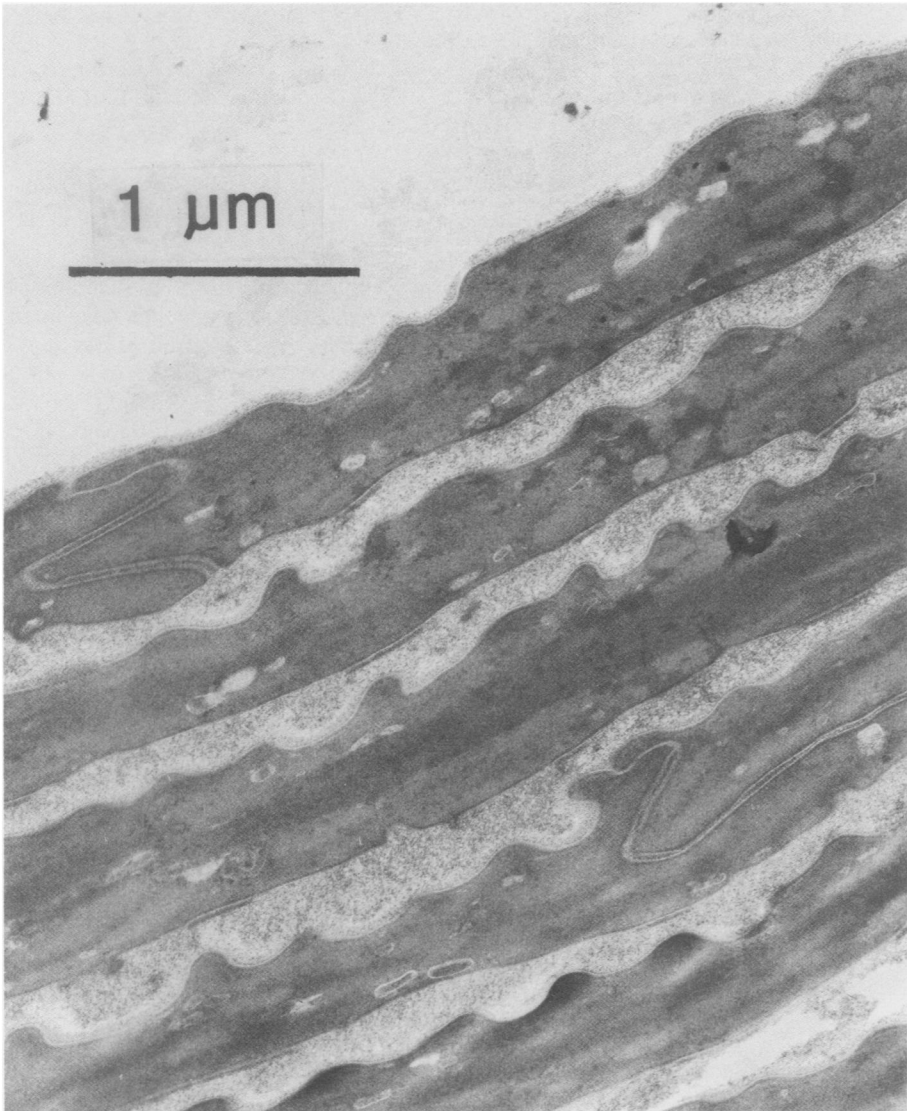


FIGURE 1. Electron micrograph of a portion of a cocoon of *Pternohyla fodiens*. Granular intercellular material in the subcorneal space separates the layers of squamous epidermal cells.

cells of the same layer, and numerous intercellular vesicles are visible. The distal surface of the cells show protruberances while the proximal surface is flat and retains desmosome fragments. The vertical thickness of the cells was approximately  $0.5 \mu\text{m}$ , and the intercellular layers about  $0.2 \mu\text{m}$ . The intercellular layer represents the original subcorneal space.

The general appearance and microscopic structure of the *Pternohyala* cocoon is very similar to the cocoon of the leptodactylid, *Lepidobatrachus* (McClanahan, et al., 1976). The epidermal layers are microscopically indistinguishable from those of *Lepidobatrachus*.

The EWL of a specimen of *P. fodiens* in flowing dry air (Table 1), after four months in a cocoon, was  $0.68 \text{ mg g}^{-1} \text{ hr}^{-1}$  (about 1.7% of body weight per day). Another measurement of EWL of this cocooned frog done 24 hrs later, yielded a similar value of  $0.62 \text{ mg g}^{-1} \text{ hr}^{-1}$ . These rates are equivalent to that of *Lepidobatrachus* per unit mass which under the same experimental conditions and after five months in a cocoon, had rates of  $0.55$  and  $1.08 \text{ mg g}^{-1} \text{ hr}^{-1}$  (McClanahan, et al., 1976). The rates per unit surface area are somewhat lower in *Pternohyala* ( $0.20 \text{ mg cm}^{-2} \text{ h}^{-1}$ ) than in *Lepidobatrachus* ( $0.37$  to  $0.73 \text{ mg cm}^{-2} \text{ h}^{-1}$ ).

TABLE 1. EWL in dry air of a specimen of *P. fodiens* in a 4 month old cocoon. Four sequential measurements at  $23^\circ\text{C}$ , BW = 11.1 g.

Time Interval min	Weight Lost mg	EWL $\text{mg hr}^{-1}$	$\text{mg g}^{-1}\text{hr}^{-1}$
105	15.9	7.9	0.71
100	14.6	9.0	0.81
125	14.0	6.5	0.59
85	7.8	5.7	0.51

## DISCUSSION

*P. fodiens* lives in a subtropical desert habitat and is distributed from Pima County, Arizona south through western Mexico to the states of Nayarit and Jalisco (Trueb, 1969; Duellman, 1970). The common name, burrowing tree frog, reflects its fossorial habits, in contrast to most hylid frogs which are arboreal. Unfortunately, almost nothing is known of the ecology of *P. fodiens* since the species is usually collected in ponds during the breeding congregations. Its burrowing habits are confirmed by the presence of a large spade-like tubercle on each of the hind feet.

All of the known anurans which form cocoons are species that burrow during long periods of drought: *Ceratophrys ornata* and *Lepidobatrachus llanensis* from the semi-arid regions of northern Argentina (McClanahan, et al., 1976); *Cyclorana alboguttatus*, *C. platycephalus*, *C. australis*, *Lymnodynastes spenceri*, and *Neobatrachus pictus* in Australia (Lee and Mercer, 1967); *Pyxicephalus adspersus* and *Leptopelis bocagei* in South Africa (Loveridge and Craye, 1979); and now *Pternohyala fodiens* in North America. All of the South American and Australian species are members of the Leptodactylidae. *Pyxicephalus adspersus* is a peculiar African form taxonomically included in the Ranidae. *P. adspersus* resembles the South American members of the genus *Lepidobatrachus* in its external morphology and aggressive behavior. *Leptopelis bocagei* is a ranoid, placed in a separate family the Hyperoliidae. The discovery of cocoon formation in *Pternohyala fodiens* extends this characteristic to the family Hylidae—an unexpected find considering the usual arboreal nature of most of the members. The cocoon of *Pternohyala* suggests that this ability may be more widespread among estivating anurans than is currently recognized.

Mayhew (1965) reported that *Scaphiopus couchi* produced a dark keratinous layer on its surface while burrowed and suggested that the covering might serve to reduce water loss. McClanahan (1967) reported similar patches of black dried skin in *S. couchi* burrowed in the laboratory. Later authors have cited these as evidence of cocoon formation in this species. However, there is no evidence that *S. couchi* (or any other species of *Scaphiopus*) forms a true cocoon. We have dug up numerous estivating specimens of *S. couchi*, *S. bombifrons* and *S. multiplicatus* and the dark covering, when present, is always in small patches and never forms a continuous envelope. The dark covering found in *Scaphiopus* resembles the skin fragments that have been described in hypophysectomized anurans (Larsen, 1976) and the fact that it is

pigmented distinguishes it from cocoons. It is possible that these skin fragments may reduce the surface area of exposed skin, but its contribution in reducing evaporative water loss is probably minimal. Furthermore direct comparisons of EWL between *S. couchi* and the cocoon forming *L. llanensis* have been made after allowing the frogs to remain burrowed for 44 days (McClanahan et al., 1976). At the end of the test period the blood plasma concentration of *S. couchi* was higher than in *L. llanensis*.

Field observations (Ruibal, et al., 1969) and laboratory experiments (McClanahan, 1967) have demonstrated that *S. multiplicatus* and *S. couchi* do not remain permanently quiescent after burrowing. During the period of estivation the toads may burrow and change the depth at which they are buried. Such activity would be impossible for a frog in a cocoon, and more importantly, would prevent the formation of an intact epidermal cocoon.

During the normal shedding (moulting) cycle of anurans the intercellular subcorneal space between the surface layer (stratum corneum) and the underlying replacement layer become enlarged and filled with a granular material (Farquhar and Palade, 1965; Budtz and Larsen, 1975). This material is apparently mucus and is secreted by the epidermal cells (Parakkal and Matoltsy, 1964). According to Whitear (1977) the mucus is secreted in the subcorneal space about 30 hours before moulting. During moulting the desmosomal connections between the cells of the stratum corneum and the replacement layer are broken and the desmosome fragments adhere to the shed stratum corneum (Budtz and Larsen, 1975). The electron-micrographs of the cocoons of *Pternohyala* and *Lepidobatrachus* demonstrate that the cocoons are composed of multiple sheddings. Each layer is a typical one cell thick stratum corneum with adhering desmosomes, dense cytoplasm, and a granular subcorneal space.

Moulting, the loss of the surface layer, is arrested by hypophysectomy (Budtz, 1977) and toads so treated will develop a multiple layered stratum corneum. Consequently, one is tempted to ascribe to the hypophysis some role in cocoon formation. However, it is possible to explain cocoon formation without invoking hypophyseal involvement. One of the characteristics of specimens of *Pternohyala* and *Lepidobatrachus* during the formation of the cocoon is the immobility of the toads. Normal moulting in many anurans involves complex and coordinated movements that bring the shed skin to the mouth where it is swallowed (Larson, 1976). Cocoon formation, at least in its initial stages requires, and may be induced by immobility. This immobility allows the detached stratum corneum to remain in place and the next, and subsequent sheddings, will also remain as envelopes and adhere to each other due to the secreted subcorneal mucus that is so evident in the electron-micrographs of sections of cocoons (Fig. 1).

Direct evidence of the immobility of *L. llanensis* during cocoon formation has recently been obtained. A time lapse film of cocoon formation during a 30-day period has been made by Ken Middleham. Using a time interval of 30 minutes only minor limb motions and few body movements were seen in the film. On moistening the frog with water it shed and swallowed the cocoon as a single piece.

The adaptive advantage of the anuran cocoon is obvious—it reduces EWL to very low levels. It can be presumed that the cocoon retards EWL because the multiple layers of dry skin passively act to increase the diffusion path of molecules and thus slow the rate of evaporation. Recently, Roberts and Lillywhite (1980) have identified a lipid component in reptilian stratum corneum as responsible for the low water permeability of reptiles. Anuran cocoons have not been tested to determine if they contain a similar "lipid-keratin" complex.

It should be emphasized that in nature the rates of EWL are certainly lower than rates determined in the laboratory. The experimental conditions of flowing dry air are much more severe than the cocooned toads would encounter while burrowed. Furthermore, the relative impermeability of the cocoon protects the frog from the desiccating effects of the increasing soil moisture tension as the soil dries. In contrast, burrowing anurans which do not have a cocoon (*Scaphiopus* and *Bufo*) are affected by the dryness of the surrounding soil. In such species water may move from the toad to the soil and the rate of this loss is partially determined by the soil moisture tension (Ruibal,

et al., 1969). In cocoonless species water uptake from the soil may also take place. Consequently as the dry season progresses, specimens of *Scaphiopus* are capable of burrowing to a greater depth and encountering soil with a higher moisture content. It appears as if two different strategies are involved:

1. Cocooned toads sealed from any appreciable exchange with the surrounding soil and remaining inactive while burrowed.
2. Toads without a cocoon and subject to water loss and gain from the surrounding soil and active while burrowed.

It can be postulated that cocoon forming species should be shallow burrowers in contrast to *Scaphiopus* and *Bufo*. It is obvious that deep burrowing is also essential where soil temperatures drop below freezing. Consequently, cocoon forming, shallow burrowing toads may be restricted to subtropical and tropical habitats. Unfortunately, we know very little about the ecology of most burrowing anurans.

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