

DENTAL DEVELOPMENT OF *Didelphis albiventris* (MARSUPIALIA). I - INCISORS AND CANINES

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ABSTRACT

The formation of incisors and canines in marsupials of *D. albiventris* was studied at various stages of development. Seventy-six specimens, with ages varying from 0 to 100 days, were used in this investigation. Serial sections of the maxilla were obtained in the transverse plane and stained with hematoxylin and eosin. Histological analyses were made to verify the pattern of teeth development, as well as their chronology of eruption. The period of time from birth to 100 days comprised the entire process of teeth development, from epithelial bud formation to early eruption of the teeth. Oral epithelium thickening gave rise to the functional incisors and canines. In addition, a secondary dental lamina emerged in different phases of development in the outer epithelium of incisors and canines, which degenerated when it reached the bud stage. No evidence of deciduous dentition was observed. The results of this investigation suggest that secondary dental lamina represents remnants of a primitive condition in which secondary dentition used to be present.

Keywords: *Didelphis albiventris*, dental development, incisors, canines.

RESUMO

Desenvolvimento dental de *Didelphis albiventris* (Marsupialia). I. Incisivos e caninos

Estudou-se o desenvolvimento dos dentes incisivos e caninos em 76 amostras de *Didelphis albiventris* com idade entre 0 e 100 dias. Cortes transversais, seriados de 6 µm de espessura foram obtidos da região da maxila, corados com Hematoxilina e Eosina e analisados ao microscópio de luz. Verificou-se que o período estudado abrange todo o desenvolvimento dental, desde a fase de iniciação da interação epitélio/mesenquima até a completa formação e erupção dos incisivos e caninos. O espessamento do epitélio oral dá origem aos incisivos e caninos funcionais, enquanto o epitélio dental externo do órgão dental origina uma lâmina dental secundária, a qual sofre degeneração, quando o dente alcança o estágio de botão. Não há vestígios de dentição decídua. Sugere-se que a lâmina dental secundária é remanescente de uma condição primitiva na qual ocorria dentição secundária.

Palavras-chave: *Didelphis albiventris*, desenvolvimento dental, incisivos, caninos.

INTRODUCTION

Teeth, like other organs, arise from a reciprocal interaction between the epithelium and the mesenchyme, which promotes cellular organization and differentiation, resulting in histogenesis and organogenesis. A continuous epithelium/mesenchyme interaction occurs during tooth development, making this organ one of the best models for differentiation studies. However, the morphogenesis of organs requires precise control of cell proliferation and differentiation in time and space. This intricate control of patterns and shapes during organogenesis is clearly evidenced in the evolutionary diversity of shapes found in mammalian molars (Jernvall, 1995; Jernvall *et al.*, 1996).

Multiple signaling molecules are expressed in the developing tooth germ and the interaction among these molecules mediates and stimulates tissue interactions. Signaling molecules belonging to the TGF β super family (BMPs, FGF, WNT and HH families) are evidenced in tooth germs at different stages and act as morphogenetic signals mediating the different steps of inductive interactions (Thesleff & Sahlberg, 1996).

A problem frequently encountered while attempting to link differences in gene expression developmental patterns bearing different morphologies is the complexity of the morphological patterns. This complexity can be a challenge when analyzing gene expression data, as well as inferring patterning mechanisms involved in morphological evolution (Gilbert & Sarkar, 2000; Weiss & Fullerton, 2000).

The teeth of modern mammals have evolved from the presumed ancestral dentition comprising three incisors, one canine, four premolars, and three molars in each dental quadrant, which appeared in two successive generations (Stock *et al.*, 1997). Mice have only one generation of functional teeth, which are reduced in number, morphologically specific and highly specialized. This remarkable specialization is an advantage of the mouse odontogenesis model.

The *Didelphidae* family is a highly interesting group because of its biological characteristics. The semi-embryonic state of the marsupial at birth provides the greatest research potential for studying tooth formation, offering one of the few

opportunities of this process for direct observation among mammals without a placental barrier and with minimal maternal metabolic influence (Jurjeski, 1974). In *Didelphis*, true organogenesis takes place during the last 3.5 days of pregnancy, corresponding to the phase in which vitelline placenta associates with uterine epithelium (Krause & Cutts, 1986).

Marsupials have been considered excellent models for ontogenetic studies owing to their short intra-uterine development. The major part of their embryogenesis occurs during the lactating period, which, in *D. albiventris*, lasts about 3 months. During the first 90 days of life, the young animal is attached to the mother's nipple in the pouch, enabling its free manipulation without surgical intervention.

However, interpretation of marsupial dentition is complicated by the presence of small, orally positioned, and non-functional teeth in relation to the functional incisors and canine during the early stages of tooth development. The homology of the so-called "prelacteal teeth" is a source of controversy in the literature (Leche, 1895). Understanding of Marsupial dentition is further complicated by the presence of lingual downgrowths of epithelium located next to the developing teeth. The meaning of these lingual downgrowths is also a subject of controversy. Some authors, such as Kukulental (1891), regarded them as representatives replacing series of teeth, while others (Wilson & Hill, 1897) consider the downgrowths a residual dental lamina.

Although several biological aspects of *Didelphis* have been studied, reports on tooth development and chronology of eruption in *Didelphis albiventris* are scanty. Therefore, the purpose of this study is to establish the chronology of development for incisors and canines in this species and to check for the presence of a successive dentition as the first step in using *Didelphis albiventris* as a model in signaling pathways during epithelium/mesenchyme interactions.

MATERIAL AND METHODS

Seventy-six (76) newborn and young opossums (*Didelphis albiventris*) from 0 to 100 days of age were used in this study (Table 1). Pregnant females were captured in their natural

TABLE 1
Specimens collected at each age (in days).

AGE	0	1	2	3	4	5	6	7	8	9	10	14	20	30	40	50	60	70	80	90	100
N	15	3	2	3	2	2	2	3	3	2	5	3	4	6	3	3	3	3	3	3	3

habitat and maintained in captivity until the animals were born. The specimens were then collected according to Table 1, removed from their mothers' nipples in the pouch, weighed and measured, anesthetized with ether, and decapitated. The heads of the specimens were fixed in Bouins fluid for 24 h at room temperature. After fixation, the heads of the animals between 1 and 15 days of age were prepared for histological evaluation without decalcification. However, the heads of the marsupials over fifteen days of age were decalcified using Planck Rychols solution for one week (Maeda *et al.*, 1986).

All the specimens were dehydrated using graded ethanol solutions, embedded in paraffin, and serially sectioned at 6 μ m in the transverse plane. Lastly, the slides were stained with H.E. or Gomori Trichromic.

All the procedures performed in this experiment were previously approved by the Brazilian Institute of Natural Environment and Renewable Resources ("Instituto Brasileiro de Meio Ambiente e de Recursos Naturais Renováveis" – IBAMA, permit # 076/94 and 076/95).

RESULTS

Dental lamina formation is one of the first events to take place during tooth development. This process begins with a thickening of the oral epithelium invaginating in the underlying mesenchyme. The five upper incisors of *D. albiventris* were found to originate from two tooth-forming areas of these invaginations. Both first and second upper incisors derived from an anterior area, which was small and narrow. In contrast, all other incisors originated from a posterior area, which was larger and expanded. Between the 6th and 7th day subsequent to birth, the third, fourth and fifth upper incisors were bud-shaped and showed individual dental laminae connecting them to the oral epithelium (Fig. 1). Inversely, each of the lower incisors presented not only its own forming area but also its own dental lamina. The lower first incisor

showed its concavity turned towards the midline until it reached the bell stage, and the secondary dental lamina was visible as a thickening of the outer epithelium (Fig. 2). A thin to moderately thick layer of dentin and enamel, located between the epithelial cap and the underlying stroma cell, was visible by day 40, when the incisors presented their definitive orientation (Fig. 3).

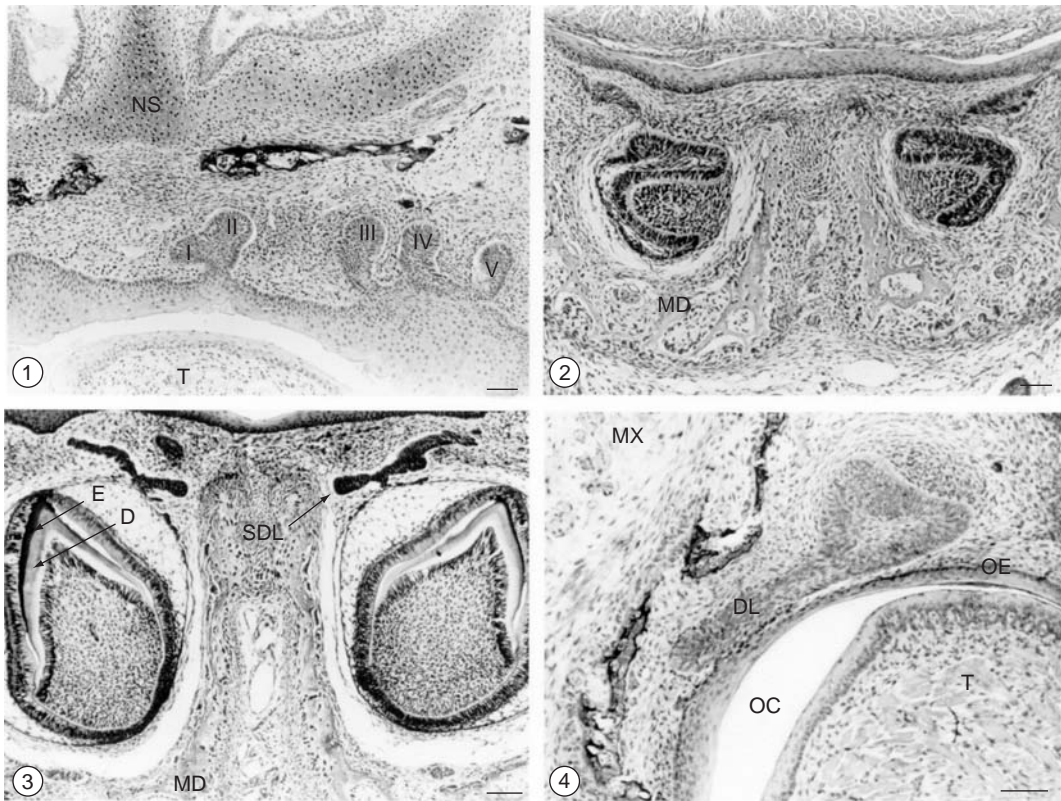
The dental lamina of the upper incisors underwent a degenerative process on the 14th day, whereas that of the lower incisors initiated this process on the 13th day. Finally, the developing teeth became fully disconnected from the oral epithelium on the 19th day. However, all the incisors showed a secondary dental lamina that arose at different stages and began to degenerate after reaching the bud stage (Fig. 3).

Canine teeth development occurred faster than other teeth, except for the lower first molar and upper second molar, which unfolded simultaneously. The upper canine dental lamina was thicker than that observed during the development of other teeth, and was positioned obliquely in relation to the oral epithelium (Fig. 4). Both dental lamina and secondary dental lamina degenerated on the 30th day. The canines were also the first teeth to reach the oral epithelium (Fig. 5), which took place around day 70.

Dentin and amelogenesis were observed in the young animals inside the pouch at about 20 days of age for canines and at 30 days for third incisors. However, first, second, fourth and fifth incisors were visible 30 to 40 days after birth (Tables 2 and 3).

Tables 2 and 3 show the histological stages of developing incisors and canines in *Didelphis albiventris* according to the following developmental stages:

- I) Dental lamina;
- II) Bud shaped;
- III) Intermediate phase between bud and cup stages;
- IV) Cup shaped;



Figs. 1-4 — 1) Frontal section through the maxillary process of 6-day-old animals. The first (I) and second (II) incisor germs were visible in the same tooth-forming area. The third (III), fourth (IV) and fifth (V) incisors showed individual dental lamina connecting them to the oral epithelium. Nasal septum (NS); Tongue (T). Bar = 100 μ m. H.E; 2) Frontal section through the mandibular process of 20-day-old animals. Note the first (I) incisor germ in the bell stage, showing its concavity turned towards the midline. Mandibule (MD). Bar = 100 μ m. H.E; 3) Frontal section through the mandibular process at 40 days of age. The first (I) incisor in the crown formation stage showed a thin to moderately thick layer of dentin (D) and enamel (E). The secondary dental lamina (SDL) arose in the outer epithelium (OE), Mandibule (MD). Bar = 100 μ m. H.E; and 4) Frontal section through the maxillary process of 7-day-old animals. Note the upper canine germ in the cap stage, showing a dental lamina (DL) obliquely positioned in relation to the oral epithelium (OE). Oral cavity, Tongue (T), MX – Maxillar. One-day-old animals. Bar = 100 μ m. H.E.



Fig. 5 — Frontal longitudinal section through the completely formed upper canine, showing its proximity to the oral epithelium (OE). Forty-day-old animals. Bar = 100 μ m. H.E.

TABLE 2
Dental development stages in upper incisors and canines from *D. albiventris*.

	First incisor	Second incisor	Third incisor	Fourth incisor	Fifth incisor	Canine
Day 0	I	I	I	I	I	I
Day 1 to 5	I	I	II	II	II	III
Day 6 to 7	II	II	II	II	II	IV B
Day 8	II	II	III	III	III	IV C
Day 10	II	III	IVB	IVB	IVB	VI A
Day 14	II	IV A	IV C	IV B	IV C	VI A
Day 20	IV A	IV A	IV C	IV C	IV C	VII B*
Day 30	IV A	IV C	VII A*	VI B*	IV C	VIII A
Day 40	VII C*	VII C*	VII C	VII C	VII C*	VIII B
Day 50	VIII A	VIII A	VIII A	VIII A	VIII A	VIII B
Day 60	VIII A	VIII C	VIII A	VIII A	VIII A	VIII C
Day 70	VIII B	VIII C	VIII D	VIII D	VIII D	X
Day 80	VIII E	X	X	X	X	X
Day 90	X	X	X	X	X	X
Day 100	X	X	X	X	X	X

*Animal's age, tooth, and developmental stage in specimens where the secondary dental lamina was observed.

TABLE 3
Dental development stages in lower incisors and canine from *D. albiventris*.

	First incisor	Second incisor	Third Incisor	Fourth incisor	Canine
Day 0	I	I	I	I	I
Day 1 to 5	II	II	II	II	III
Day 6 to 7	II	III	III	III	IV B
Day 8	III	III	III	III	IV C
Day 10	IV B	IV A	IV B	IV B	V
Day 14	V	IV C	V	IV C	VI A
Day 20	VI A	IV C	VI A	IV C	VII B*
Day 30	VII C*	VII B	VII C*	VII B*	VII B**
Day 40	VII C	VII C*	VII C	VII C	VII C
Day 50	VIII A	VIII A	VIII A	VIII B	VIII A
Day 60	VIII C	VIII C	VIII D	VIII D	VIII C
Day 70	VIII E	VIII E	VIII E	VIII E	X
Day 80	X	X	X	VIII E	X
Day 90	X	X	X	X	X
Day 100	X	X	X	X	X

* Animal's age, tooth, and developmental stage in specimens where the secondary dental lamina was observed

** Animal's age, tooth, and developmental stage which the secondary dental lamina with bud tip was present.

- IVa) Nondifferentiated dental organ;
 IVb) Dental organ in differentiation ; and
 IVc) Differentiated dental organ.
- V) Intermediate stage between cap and bell stages;
- VI) Bell shaped;
 VIa) Dental lamina connected to the oral epithelium;
 VIb) Dental lamina in degenerative process;

- VII) Crown formation;
 - VIIa) Dentinogenesis ;
 - VIIb) Dentinogenesis and initial signs of amelogenesis at the tip of the tooth;
 - VIIc) Dentin formation completed and enamel present on at least 1/3 of the tooth crown;
- VIII) Rizogenesis stage;
 - VIIIa) Epithelial root sheath without odontoblast differentiation;
 - VIIIb) Epithelial root sheath with odontoblast differentiation;
 - VIIIc) Pre-dentin formation;
 - VIIIc) Dentin formation;
 - VIIIe) Cement and periodontal fibers;
- IX) Tip of the cuspid reaches oral epithelium;
- X) Eruptive stage: tooth already seen in the oral cavity.

DISCUSSION

Most dental development studies reported in the literature estimated the age of animals based on growth lines, according to the specimen's body weight and length. Weight is the parameter which presents the largest standard deviation, regarding its inverse relation to the number of animals by brood (Dezone *et al.*, 1984). The ages of all specimens of *Didelphis albiventris* used in this study were recorded. Histological evaluations were made of serial sections of the dental arches from each age group studied.

The dental formula of adult *Didelphis albiventris* is:

$$\begin{array}{ccccc} 5 & 1 & 3 & 4 & \\ I & C & P & M & \\ 4 & 1 & 3 & 4 & \end{array}$$

Before tooth development in *D. albiventris*, two oral epithelium thickenings invaginated into the underlying ectomesenchyme of the maxillary region, and all the functional upper incisors developed from these invaginations. Both first and second maxillary incisors (I^1 and I^2) originated from the anterior thickened area. However, the posterior region of the invaginations gave rise to third, fourth and fifth maxillary incisors (I^3 , I^4 and I^5). Conversely, each of the four incisors in the lower jaw had its own forming area and its own dental lamina. These findings are similar to those observed in other marsupials.

Setonix brachyurus also showed also two tooth-forming areas. The anterior tooth-forming area was subdivided into two regions: the first located anteriorly, and the second posteriorly. The first area was smaller and narrower, giving rise to the first non-functional maxillary incisor (i^1). Inversely, the second region was larger and lingually expanded, thus originating the maxillary first functional incisor (I^1). The posterior tooth-forming area consisted of three thickenings: an anterior thickening, which gave rise to the second functional incisor (I^2); a mid-buccal one, from which the second non-functional incisor developed (i^2); and a posterior one, which originated the third functional incisor (I^3). According to their buccal or lingual positions, and their early development and calcification, non-functional teeth formed a primary series of teeth, while the functional dental elements comprised a secondary or permanent series (Berkovitz, 1968). In *P. eugenii*, two non-functional incisors (i) were buccally positioned in relation to the functional incisors (I) in both jaws. They also developed and calcified before the functional dental elements (Berkovitz, 1972).

In *S. virginiae*, the formation of small epithelial cap-nodules at the end of the short primary dental lamina stalks has been reported in 5 to 10-day-old animals in the pouch. These structures gave rise to the mandibular first and third deciduous incisors ($dI_{1,3}$), as well as to the deciduous canine (dC). A notable improvement in differentiation and formation of a thin to moderately thick dentinal arc was observed at around the 10th day. This structure was located between the epithelial cap and the underlying mesenchyme cells of the lower second deciduous incisor (dI_2). Between the 25th and 30th day, this dentinal arc was still associated with the small dI_2 cap, whereas the deciduous rudiments were less distinct for most of the anterior teeth in both jaws (Luckett & Woolley, 1996).

The rudimentary lower second deciduous incisor (dI_2) was not evident in 55 to 60-day-old animals in the pouch, presumably because the mandibular first and second permanent incisors ($I_{1,2}$) were in an early phase of eruption through the oral epithelium, and the tiny superficially placed dI_2 was probably shed and lost during the eruptions of the successor teeth.

Dentin formation initiated on day 20 for canines, on day 30 for third incisors and between the day 30 and 40 for the other incisors. In contrast, while studying incisor development in *P. eugenii*, Berkovitz (1972) noted that dentinogenesis occurred on day 52.

According to the findings of this investigation, between days 0 and 100, all five incisors and canines were completely developed and were visible in the oral cavity of both jaws. The earliest penetration of the oral epithelium for the canine was on around day 70, followed by second, third, fourth and fifth incisors on day 80. The first and the fourth incisors were the last teeth to erupt in the maxilla and in the mandible, respectively (Table 3). These observations are in agreement with the results reported by Luckett & Wolley (1996), who described incomplete first incisor eruption in *Sminthopsis virginiae* between days 79-81.

In *Perameles gunii*, it has been noted that two incisor germs did not develop in the lower jaw. Instead, they degenerated and were considered the so-called "absorptive teeth". Inversely, all five incisors developed in the maxilla and were considered part of the deciduous dentition (Wilson and Hill, 1897). Dependorf (1898) considered the "absorptive teeth" part of a pre-lacteal dentition, whereas Müller (1969) suggested they were part of the same dentition as the other teeth. While studying *Didelphis albiventris* and *S. brachiurus*, Kühental (1891) and Berkovitz (1966), respectively, found no absorptive teeth. In fact, they found the dental laminae were positioned lingually to the developing tooth germs. The former author believed these teeth were remnants of the secondary dentition, while the latter interpreted them as remnants of the dental lamina free edge rather than a substitutive set of teeth.

The genus *Didelphis* has conserved the dental formula of its ancestral marsupials, while other species of these animals have undergone different reductions in their dental number. The authors of the present study support the theory that the non-functional absorptive teeth and the pre-lacteal dental elements are phylogenetic degeneration processes of the original teeth, as reported by Leche (1895), Wilson & Hill (1897), Dependorf (1898), and Muller (1969).

Gray short-tailed opossums (*Monodelphis domestica*) reportedly exhibited monophyodonty

and have about 50 teeth (dental formula: 5134/4134). In these animals, the tooth germ of the maxillary and mandibular deciduous canines, as well as the deciduous maxillary first incisor, had a successor dental lamina and a replacement tooth germ. Tooth germs of both deciduous mandibular first incisor and deciduous maxillary fourth incisor had developed up to the bud stage by day 12, but were reduced by day 18 (Kozawa et al., 1998).

The results of this study showed that thickening of the oral epithelium (primary dental lamina) gave rise to the functional incisors and canines in *D. albiventris*. In addition, a secondary dental lamina emerged in different developmental stages in the outer epithelium of the incisors and canines, undergoing degeneration when it reached the bud stage. These observations suggest that the first dentition prevailed as the permanent dentition. The findings of the present investigation are congruent with those reported by Kukental (1891) and Fosse (1994), who described these dental laminae as remnants of a primitive condition when secondary dentition used to be present.

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