

**MODELLING THE SPATIAL PATTERNING OF
THE TEETH PRIMORDIA IN THE LOWER JAW
OF *Alligator mississippiensis***

P. M. KULESA¹, G. C. CRUYWAGEN², S. R. LUBKIN³, P. K. MAINI⁴,
J. SNEYD⁵ and J. D. MURRAY³

¹*Division of Biology, Beckman Institute (139-74),
Caltech, Pasadena, CA, 91125, USA*

²*Sea Fisheries Research Institute, Private Bag X2,
Rogge Bay, 8012 Capetown, SA*

³*Department of Applied Mathematics, University of Washington,
Box 352420, Seattle, WA, 98195-2420, USA*

⁴*Centre for Mathematical Biology, Mathematical Institute,
24-29 St Giles', Oxford OX1 3LB, UK*

⁵*Department of Mathematics, University of Canterbury,
Private Bag 4800, Christchurch, NZ*

ABSTRACT

We propose a model mechanism for the initiation and spatial positioning of teeth primordia in the alligator, *Alligator mississippiensis*. Detailed embryological studies¹²⁻¹⁴ have shown that jaw growth plays a crucial role in the developmental patterning of the tooth initiation process. The development of the spatial pattern occurs on a timescale comparable to jaw growth. Based on biological data we develop a dynamic patterning mechanism, which crucially includes domain growth. The mechanism can reproduce the spatial pattern development of the first seven teeth primordia in the lower jaw of *A. mississippiensis*. The results for the precise spatio-temporal sequence compare well with experiment.

Keywords: Alligator, tooth initiation, pattern formation, model.

1. Introduction

The developmental processes involved in the embryonic vertebrate jaw offer a system which has the aspects of self-organization, pattern formation, tissue interaction and growth. Although analysis of the vertebrate jaw has been extensive, no clear mechanism(s) has emerged to explain the developmental processes of tooth initiation, tooth shape or palate formation. We are particularly interested in the spatial patterning of the teeth primordia that occurs in the tooth initiation process.

Experimental investigations of mammalian dentition development have been hindered by the inaccessibility of detailed embryonic processes *in vivo*. This problem can be overcome by studying dental embryology in the crocodylia, in particular *Alligator mississippiensis*, which exhibits the unique combination of developing in

an external egg and possessing the most mammal-like snout and palate of all the crocodylia [2]. An understanding of the basic developmental mechanisms involved in crocodylian embryology may provide clues to the morphogenesis of advanced structures found in higher vertebrates [2].

The origin of embryological investigations of dentition development in reptiles [10,15,7] forms the basis for descriptive models of tooth formation, which in general fall into either a prepattern (the pattern is imposed from an external source) or dynamic (arises as part of the dynamics of the developing system) model category. Edmund's [1] Zahnreihe theory postulated that initiation waves from the front of the jaw activated prepatterned tooth sites. Osborn [8] proposed the clone model which postulates that teeth are initiated from one or more clones of neural crest cells that form pattern as a result of the dynamic growth of the clone. Osborn [9] has recently extended this clone model to incorporate tooth shape development.

More recently, a series of detailed experiments on the embryonic development of the lower jaw [12,13] and upper jaw [14] dentition of *Alligator mississippiensis* has been completed from day 1 to day 75; it has a 65-day incubation period. Their data show that neither Zahnreihen nor the clone model can account for the spatial patterning of the teeth primordia.

We propose here a model mechanism which details the process of spatial patterning of the teeth primordia and predicts the spatial and temporal sequence of the first seven teeth in the lower jaw of *Alligator mississippiensis*, based on the data of [12]. A previous model, based on a mechanical approach, for initiation of the dental determinant has been given by Sneyd *et al.* [11].

2. The Model Mechanism

2.1. Biological Basis of the Model for Tooth Initiation

Development of an individual tooth is characterised by a series of processes, from tooth initiation to shape formation, through to development of the dentine and enamel organ. We focus on the spatial patterning of discrete primordia that result in the tooth initiation process (Fig. 1(a)).

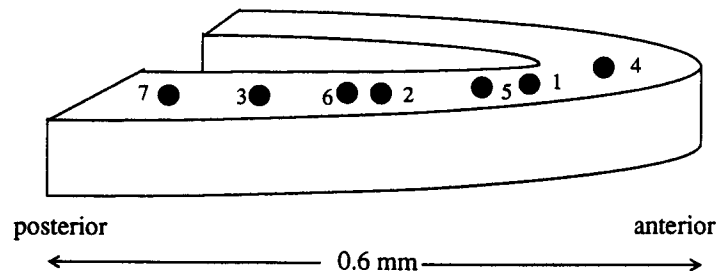


Fig. 1. (a) Spatial pattern of first seven teeth primordia in the lower right half jaw of *A. mississippiensis* (from [12]).

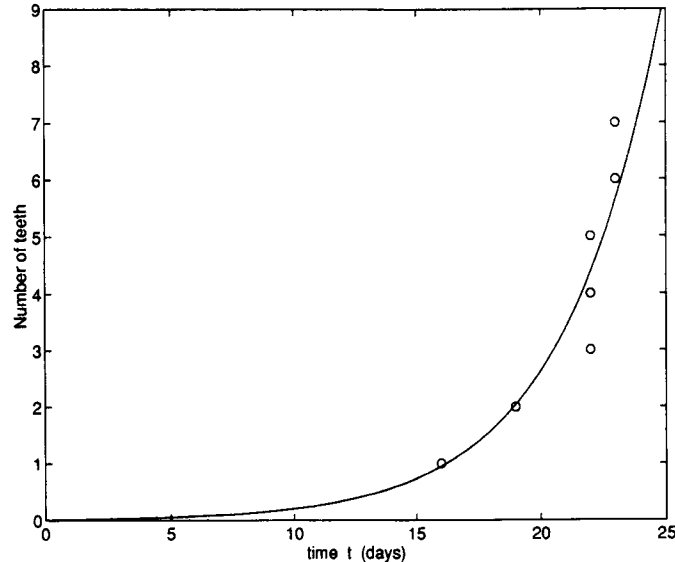


Fig. 1. (b) Temporal sequence of first seven teeth primordia with the solid line representing $N(t) = N_0 e^{rt}$; $N_0 = 0.0066$, $r = 0.31/\text{day}$ (derived from [12]).

The first stages of tooth initiation begin with the thickening of the oral epithelium which forms a localised condensation of cells to mark the first tooth site, the dental determinant. The subsequent teeth primordia form in the same manner as the dental determinant.

Experiments in mice [4,5] have shown that it is the epithelium which initiates tooth development and that an epidermal growth factor is involved in the initiation process [3]. Although experiments have not concluded such results in reptiles, it is reasonable to assume that tooth development in reptiles is also initiated by the epithelium.

In their work on alligator dentition, Westergaard and Ferguson [12] show that the dental determinant of *A. mississippiensis* forms in the anterior (front) part of the lower jaw. It is not the most anterior tooth to form. Tooth initiation spreads from the dental determinant forwards and backwards in the jaw, and interstitial teeth are formed in the growing spaces between earlier teeth. The results of the spatial and temporal sequence of the first seven teeth are shown below in Figs. 1(a) and (b).

The tooth initiation sequence is symmetric for both the right and left lower jaw halves [12,13], and we model the tooth initiation process on the half lower jaw as a one-dimensional domain, with the initiation and patterning of primordia an epithelial process. We assume the jaw growth is exponential during early dentition ([12]; Fig. 1(b)).

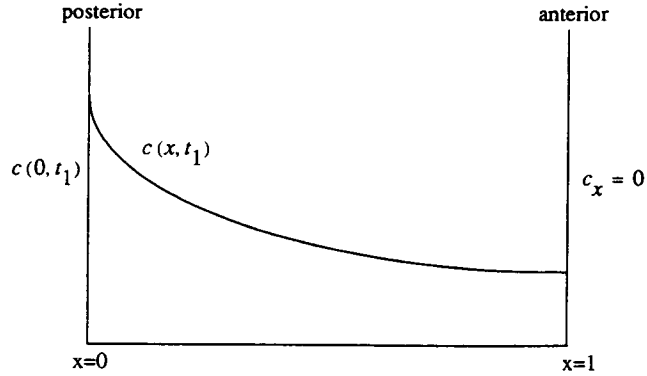


Fig. 2

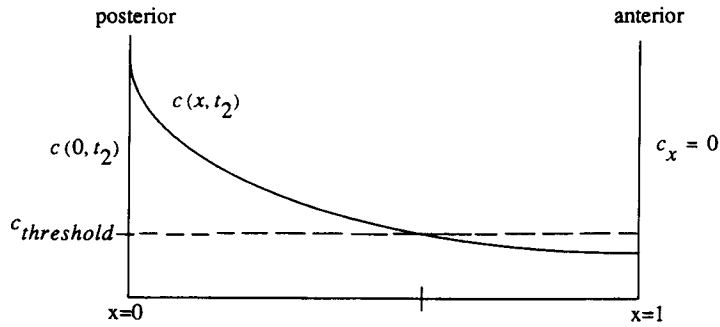


Fig. 3

2.2. How the Mechanism Works

We propose a dynamic reaction-diffusion mechanism [6], mediated by an inhibitor. The following notation is used

- $u(x, t)$ = substrate concentration at position x and time t ,
- $v(x, t)$ = activator concentration at position x and time t ,
- $c(x, t)$ = inhibitor concentration at position x and time t .

We assume that there is an initial source of inhibitor, c at the posterior end of the jaw ($x = 0$). The tooth initiation process begins as this source chemical diffuses through the jaw epithelium and degrades (Fig. 2). As the jaw grows, c decreases further towards the anterior end until it crosses below the critical threshold to drive the activator and substrate system unstable (Fig. 3).

Our conservation equation for the inhibitor, c , on a fixed domain $\xi \in [0, 1]$ is taken to be

$$\frac{\partial c}{\partial t} = \underbrace{-\delta c}_{\text{degradation}} + p \underbrace{\frac{\partial^2 c}{\partial \xi^2}}_{\text{diffusion}}, \quad (2.1)$$

where p is the diffusion coefficient and δ is the degradation constant.

As a result of the experimental evidence of jaw growth (Fig. 1(b)), we consider that a jaw segment grows at a constant strain rate, r . The growing domain dilutes the concentration, but a larger domain produces more growth factor. On a growing domain at a constant strain rate, r , but transformed ($x = \xi e^{-rt}$) to a fixed domain $x \in [0, 1]$, (2.1) becomes (see Appendix for derivation)

$$\frac{\partial c}{\partial t} = \underbrace{-\delta c}_{\text{degradation}} - \underbrace{rc}_{\text{dilution due to jaw growth}} + \underbrace{p(e^{-2rt}) \frac{\partial^2 c}{\partial x^2}}_{\text{diffusion}}. \quad (2.2)$$

When the subdomain, on which c is below the threshold, has grown large enough, a single mode spatial pattern in u and ν will start to grow (Fig. 4). When the concentration $u(x, t)$ crosses an upper threshold this triggers initiation of a placode, a tooth primordium, giving the spatial position of the dental determinant (Fig. 5).

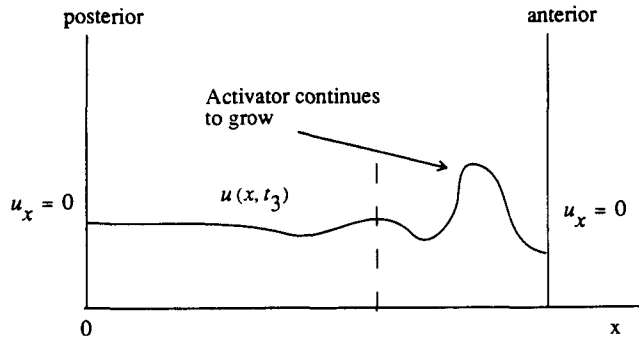


Fig. 4

We take a simple reaction-diffusion mechanism for the activator-substrate system, namely the Schnackenberg mechanism (see, for example, [6]). The equations for the substrate, u , and activator, ν , on a domain growing at a constant strain rate, r , are transformed to a fixed domain $x \in [0, 1]$ (in the same way as for (1.2)) as

$$\frac{\partial u}{\partial t} = \underbrace{\gamma[hc - u + u^2\nu]}_{\substack{\text{reaction kinetics} \\ \text{and bifurcation} \\ \text{"parameter", } c(x,t)}} - \underbrace{ru}_{\text{dilution due to jaw growth}} + \underbrace{(e^{-2rt}) \frac{\partial^2 u}{\partial x^2}}_{\text{diffusion}}, \quad (2.3)$$

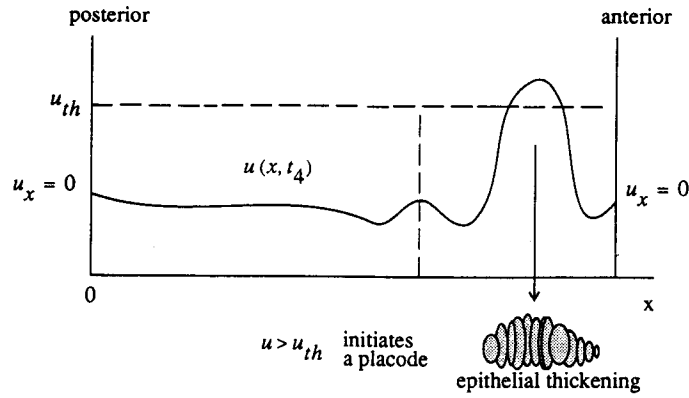


Fig. 5

$$\frac{\partial v}{\partial t} = \underbrace{\gamma[b - u^2 v]}_{\text{reaction kinetics}} - \underbrace{rv}_{\text{dilution due to jaw growth}} + \underbrace{d(e^{-2rt})}_{\text{diffusion}} \frac{\partial^2 v}{\partial x^2}, \quad (2.4)$$

where d is the diffusion coefficient ratio.

Experimental evidence suggests that the dental determinant (and each subsequent tooth primordium) becomes a source of growth factor c (simulating an inhibition zone): there are now two sources. As the jaw grows, c drops below the critical threshold in the region between the two sources and the next tooth position forms in the posterior end of the jaw (Fig. 6). The second primordium forms in the region where u again crosses the patterning threshold, and the tooth that is initiated becomes another source of c (Fig. 7).

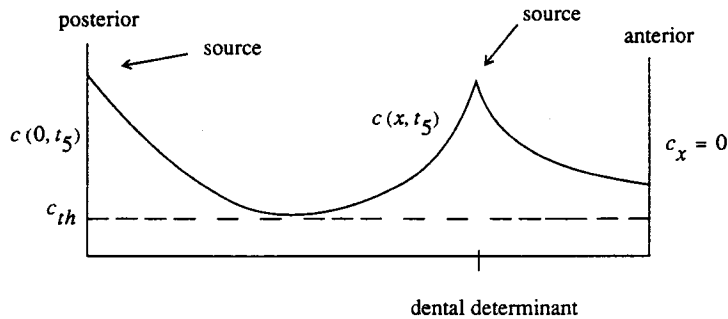


Fig. 6

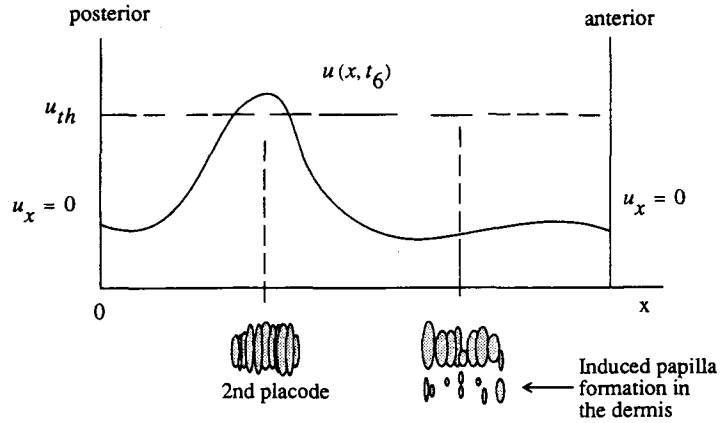


Fig. 7

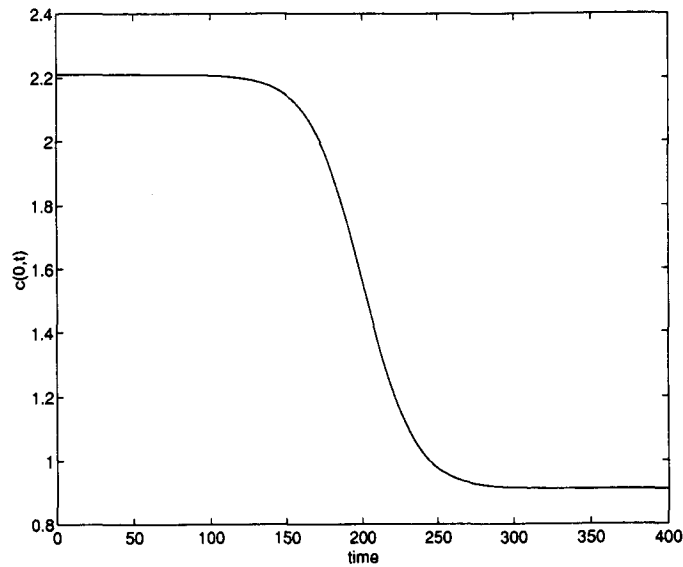


Fig. 8. Representative continuous function for source at $x = 0$, $c(0, t) = -0.65 \tanh \left[\frac{t-200.01}{34} \right] + 1.56$.

3. Numerical Simulations

The system of equations (2.2), (2.3) and (2.4) were solved numerically using a finite difference spatial discretisation and the method of lines to integrate in time (NAG: D03PCF). The structure of the simulations was to first, numerically solve for the position of tooth 1 on $x \in [0, 1]$, then partition this interval into two subdomains

and solve for the position of tooth 2. The numerical simulations for the remainder of the first seven teeth followed in this way (see Appendix).

The initial conditions for u , ν , and c were given by the previous simulation except at time $t = 0$, when $c(x, 0) = c_0(x)$, a monotonically decreasing function, and u and ν were at homogeneous steady states. All simulations were run with no flux boundary conditions on u and ν , while the boundary conditions for c varied as to whether one of the simulation domain endpoints included $x = 0$ (posterior source), $x = x_i$ (tooth position source of strength = 1), or $x = 1$ (no flux). The posterior end source, $c(0, t)$, we considered to decay with time as in (Fig. 8).

A comparison of the numerical simulation data versus the experimental data [12] of the first seven teeth is shown in Fig. 9.

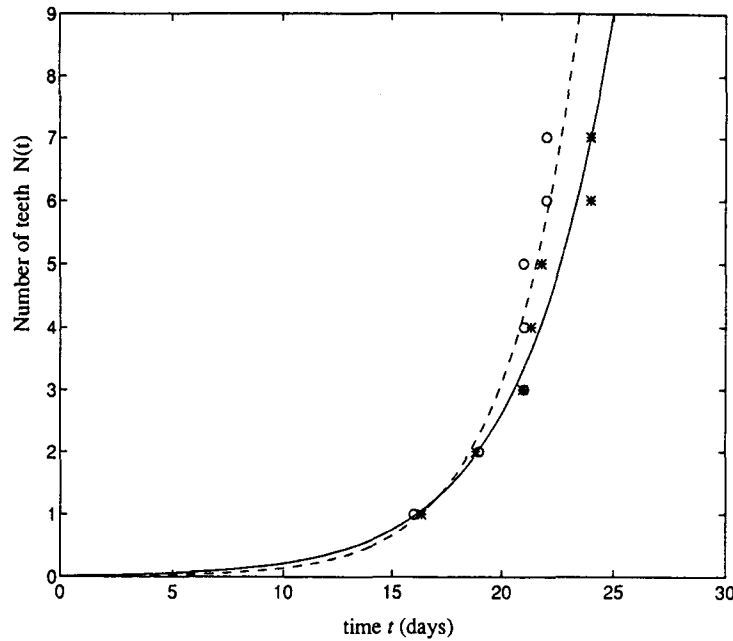


Fig. 9. Numerical vs experimental data for the first seven teeth primordia in lower right half jaw of *A. mississippiensis* (from [12]).

* = numerical data with solid line, $N(t) = N_1 \exp(r_1 t)$.

o = experimental data from Fig. 1(b) with dashed line, $N(t) = N_2 \exp(r_2 t)$

($N_1 = 0.018$, $r_1 = 0.25/\text{day}$, $N_2 = 0.0066$, $r_2 = 0.31/\text{day}$) Time, T (days) was scaled to t (simulation) using; t (simulation) = $KT + b$, $K = 23.18/\text{day}$ and $b = 225.8$.

4. Acknowledgements

This work (PMK, JS, JDM, SRL) was in part supported by grant DMS-9106848, from the U.S. National Science Foundation.

Appendix. The Effect of Exponential Growth on a Reaction-Diffusion Equation

We consider a jaw segment, $l(t)$, which grows at a constant strain rate, r , so that

$$\frac{dl}{dt} = rl \rightarrow l(t) = l_0 e^{rt}. \tag{A.1}$$

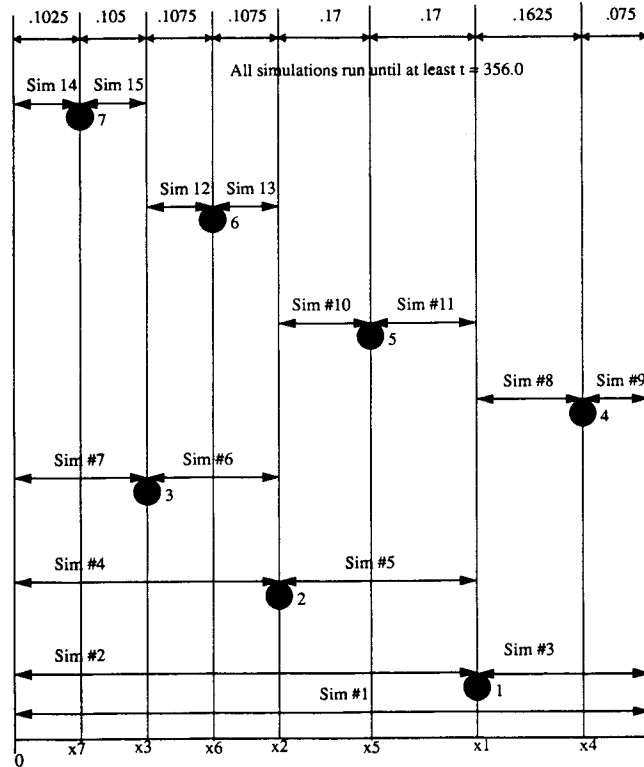


Fig. A.1. Numerical simulation domain chart. The parameters used for the simulations: $\gamma = 40.0$, $\delta = 0.2$, $h = 1.0$, $b = 2.0$, $p = 0.5$, $r = 0.01$, $d = 150$.

For a general form of (1.1) on the domain $\xi \in [0, L]$, growing at a constant strain rate, r ,

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial \xi^2} + \gamma f(c). \tag{A.2}$$

Let s equal the quantity of reactant in length l , which implies $c = s/l$. In the time interval $(t, t + \Delta t)$, the length increases from l to $l + \Delta l$ and the concentration changes from $c = s/l$, to $(s + \Delta s)/(l + \Delta l)$,

$$\begin{aligned} \Delta c &= [(s + \Delta s)/(l + \Delta l)] - s/l \\ &\approx [(s + l\gamma f(c)\Delta t)/(l + r\Delta l)] - s/l \\ &\approx \gamma f(c)\Delta t - rc\Delta t. \end{aligned} \tag{A.3}$$

which implies that $\lim_{\Delta t \rightarrow 0} [\Delta c/\Delta t] = \gamma f(c) - rc$.

The growing domain dilutes the concentration, but a larger domain produces more c . Equation (A.2) becomes,

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial \xi^2} + \gamma f(c) - rc. \quad (\text{A.4})$$

Setting $x = \xi e^{-rt}$, where x is the fixed domain variable, (A.4) becomes,

$$\frac{\partial c}{\partial t} = D(e^{-2rt}) \frac{\partial^2 c}{\partial x^2} + \gamma f(c) - rc \quad (\text{A.5})$$

which represents the equation on a domain growing at a constant strain rate, r , but transformed to a fixed domain $x \in [0, L]$.

References

- [1] Edmund A. G., Evolution of dental patterns in the lower vertebrates. In *Evolution: Its Science and Doctrine*, R. Soc. Can. Studia Varia. Ser. 4 (1960) pp. 45–62.
- [2] Ferguson M. W. J., The value of the American alligator (*Alligator mississippiensis*) as a model for research in craniofacial development, *J. Craniofac. Genet. Dev. Biol.* **1** (1981) pp. 123–144.
- [3] Kronmiller J. E., Upholt W. B. and Kollar E. J., EGF Antisense oligodeoxynucleotides block murine odontogenesis *in vitro*, *Dev. Biol.* **147** (1991) pp. 485–488.
- [4] Lumsden A. G. S., Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ, *Development 103 Supplement* (1988) pp. 155–169.
- [5] Mina M. and Kollar E. J., The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium, *Archs. Oral Biol.* **32** (1987) pp. 123–127.
- [6] Murray J. D., *Mathematical Biology* (Springer-Verlag, Heidelberg, 1989) 2nd edition.
- [7] Osborn J. W., The ontogeny of tooth succession in *Lacerta vivipara* Jacquin (1787), *Proc. R. Soc. Lond. (B)* **179** (1971) pp. 261–289.
- [8] Osborn J. W., Morphogenetic gradients: fields versus clones. In *Development, Function and Evolution of Teeth*, eds. Butler P. M. and Joysey K. A. (Academic Press, London and New York, 1978) pp. 171–201.
- [9] Osborn J. W., A Model Simulating Tooth Morphogenesis without Morphogens, *J. Theor. Biol.* **165** (1993) pp. 429–445.
- [10] Rose C., Ueber die Zahnentwicklung der Crocodile (On the tooth development of crocodiles), *Morph. Arbeit.* **3** (1984) pp. 195–228.
- [11] Sneyd J., Atri A., Ferguson M. W. J., Lewis M. A., Seward W. and Murray J. D. A model for the spatial patterning of teeth primordia in the Alligator: initiation of the dental determinant, *J. Theor. Biol.* **165** (1993) pp. 633–658.
- [12] Westergaard B. and Ferguson M. W. J., Development of the dentition in *Alligator mississippiensis*, Early embryonic development in the lower jaw, *J. Zool. Lond.* **210** (1986) pp. 575–597.
- [13] Westergaard B. and Ferguson M. W. J., Development of the dentition in *Alligator mississippiensis*. Later development in the lower jaws of embryos, hatchlings and young juveniles, *J. Zool. Lond.* **212** (1987) pp. 191–222.

- [14] Westergaard B. and Ferguson M. W. J., Development of the dentition in *Alligator mississippiensis*. Upper jaw dental and craniofacial development in embryos, hatchlings and young juveniles, *Am. J. Anatomy* **187** (1990) pp. 393–421.
- [15] Woerdeman M. W., Beitrage zur Entwicklungsgeschichte von Zahnen und Gebiss der Reptilien. Beitrag I. Die Anlage und Entwicklung des embryonalen Gebisses als Ganzes und seine Beziehung zur Zahnleiste (Contributions to the evolutionary history of teeth and dentition of reptiles. I. The layout and development of the embryonic dentition as a whole and its relation to the jaw), *Arch. Mikrosk. Anat.* **92** (1919) pp. 104–192.