Numeryczne przybliżenia nowego modelu kształtowania si wzorców kostnych

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Numerical approach to a new model of limb pattern formation

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The organization of cells and tissues into specific arrangements or patterns during embryogenesis, and the inheritance of these pattern-forming mechanisms constitute important problems of both developmental and evolutionary biology [1].
The patterning of the skeletal elements in vertebrate limbs is an experimental system within which these issues have received particular attention [2].

The quasi-periodic arrangement of limb bones is well conserved across the animal kingdom and consists of a progressive increase in element number along the proximal-distal axis [3].
Each skeletal element is preceded by a cartilage element, which in turn arises from condensations of limb mesenchymal cells [4].
The condensation of mesenchymal cells can also be observed in vitro, in so-called “micromass” cultures.

When precartilage mesenchymal cells are isolated from a developing chicken limb, dissociated and cultured at high densities on tissue culture plastic in serum-free conditions, they organize themselves into spot- or rod-like condensations of nearly uniform size and regularity of spacing surrounded by non-aggregated cells [5, 6, 7].
When packed into a limb bud ectodermal jacket the cells generate poorly formed, though discrete cartilaginous elements [8, 9].

Recently, in an attempt to clarify the identities of early-acting determinants of precartilage condensations, [10] showed that two members of a class of glycan-binding proteins called galectins appear at the sites of prospective condensation in the developing chicken limb before any previously described condensation mediators such as fibronectin.
These galectins are CG (chicken galectin)-1A and CG-8. (See also [11].)

Ectopic CG-1A induced supernumerary condensation formation in vitro and digit formation in vivo, both of which were inhibited by CG-8.
What distinguishes the interaction of these gene products from other experimentally elucidated LALI networks is a mutually positive feedback loop exerted by the proteins on each other's gene expression with the inhibitory effect exerted at a different biological level, protein-protein interaction [10].
In addition, CG-1A induces the expression of a shared counterreceptor.
A relevant question is whether the demonstrated interactions were sufficient to give rise to the characteristic condensation pattern or if additional components or interactions are required.

In [12], a mathematical model was constructed and numerically analyzed that incorporates the interactions of CG-1A and CG-8 multilevel regulatory network to explore their ability to form spatial patterns of condensations.
It was verified that this mathematical model does indeed reproduce the experimental findings, and in the process, gives rise to a condensation-like pattern.
A number of explicit predictions of the model for further experimental tests is listed in section 5 of [12].

The mathematical model is based on a number of biological assumptions concenrning the relevant molecules, the proteins chicken galectin-1A (CG-1A) and chicken galectin-8 (CG-8), which diffuse in the ECM, as well as their counterreceptors, which are membrane bound. We assume that there are two types of counterreceptors: One that can only bind to CG-8 whereas there is a “shared” counterreceptor which can bind to both CG-1A and CG-8.

We assume that it is through binding of CG-1A to the shared counterreceptor that the former enhances expression of the shared counterreceptor and of CG-8 and that the binding of CG-8 to its unique counterreceptor enhances expression of CG-1A; and finally, binding of CG-8 to the shared counterreceptor has no regulatory effect (other than the indirect one of making the binding site inaccessible to CG-1A).
We also assume that in contrast to the case with CG-1A, the binding of CG-8 to either of its counterreceptors has no effect on their expression.
The regulatory effects of binding of a galectin to a counterreceptor is summarized in Figure 2.

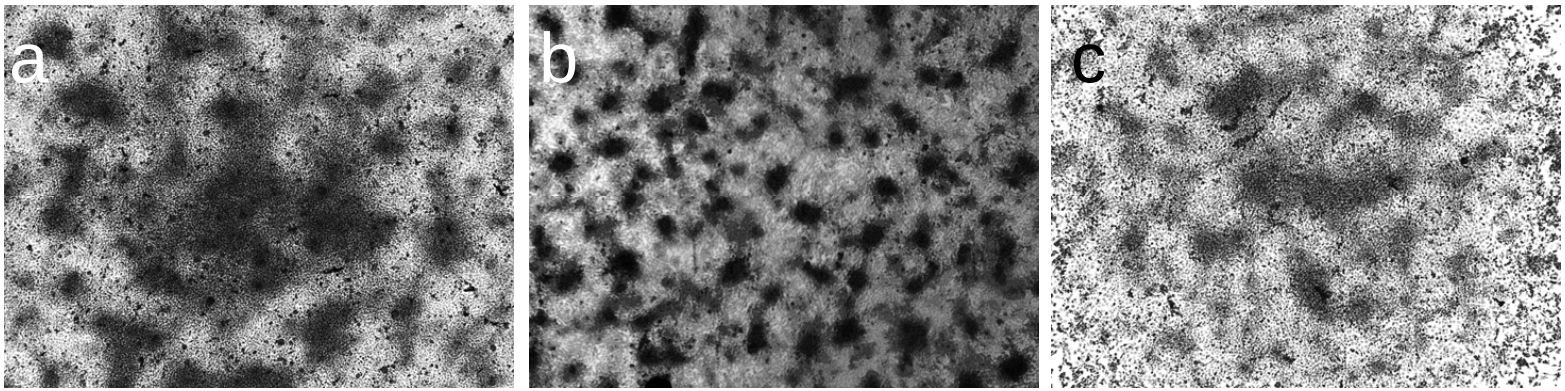

Figure: Images of chondrogenic condensation patterns of leg cultures for three cases: control (a); added CG-1A (b); and added CG-8 (c). Note that the addition of CG-1A causes increased condensation numbers, with concomitant decrease in size as compared to the control; addition of CG-8 causes fewer condensations, with with concomitant decrease in size.

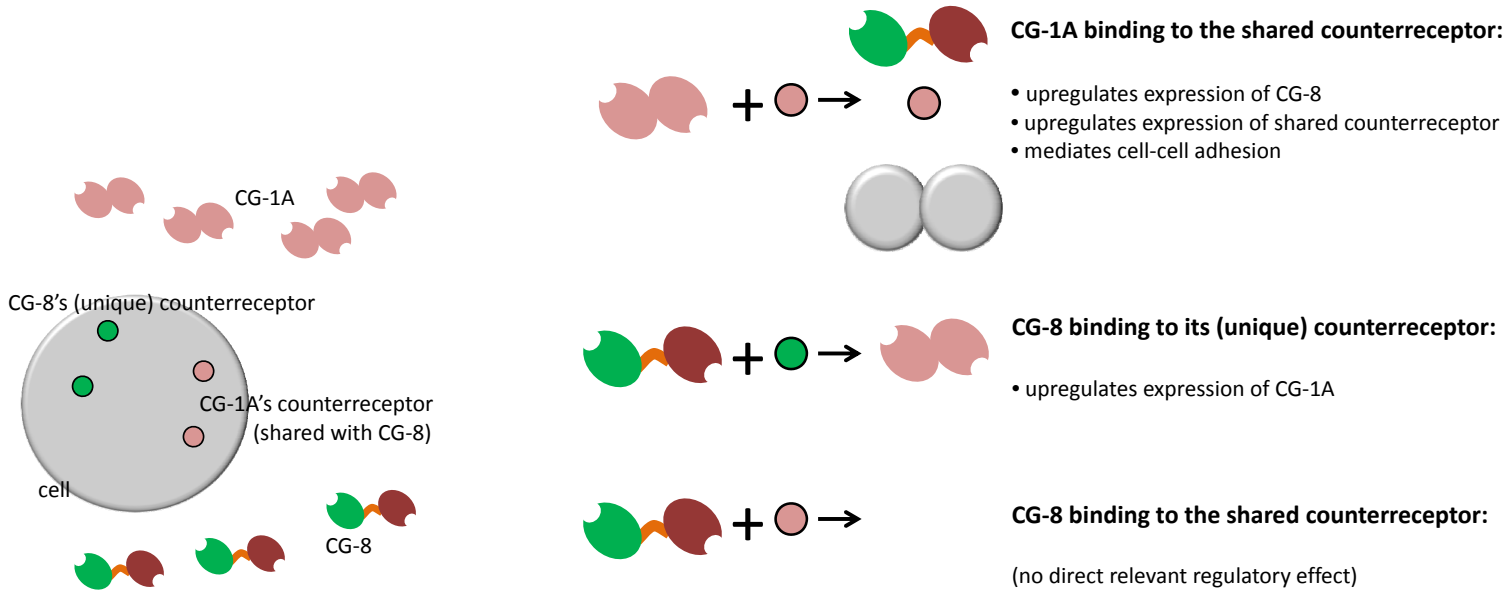

Figure: Schematic representation of the galectin regulatory network: **Left:** graphical representation of matrix-bound galectins and their cell membrane-bound counterreceptors; **right:** schematic representation of the "minimal" regulatory network described in the text: the effects of galectins binding to counterreceptors.

In this section, we describe the complete mathematical model of the pattern formation mechanism.
The relevant variables are the cell densities, the concentrations of the counterreceptors, the concentrations of the freely diffusing galectins and those bound to their counterreceptors.
Table 1 lists the variables and summarizes our notations.

t	time
$\mathbf{x}$	location
$c_1^u = c_1^u(t, \mathbf{x})$	concentration of freely diffusible CG-1A (that is, CG-1A not bound to counterreceptors on cell membranes)
$c_8^u = c_8^u(t, \mathbf{x})$	concentration of freely diffusible CG-8 (that is, CG-8 not bound to counterreceptors on cell membranes)
$R = R(t, \mathbf{x}, c_1, c_8^8, c_8^1, \ell_1, \ell_8)$	cell density w.r.t. the variables: volume,
	concentr. c_1 of CG-1A bound to shared count
	concentr. c_8^8 of CG-8 bound to CG-8's unique
	concentr. c_8^1 of CG-8 bound to shared counter
	concentr. ℓ_1 of shared counterreceptors (not b
	concentr. ℓ_8 of CG-8 counterreceptors (not bound to galectins) on cell membran

Table: List of variables used in the model.

Note that the cell density R depends on several variables representing various chemical concentrations besides time and space.
A proper mathematical viewpoint is that

$$R(t, \mathbf{x}, c_1, c_8^8, c_8^1, \ell_1, \ell_8) d\mathbf{x}\, dc_1\, dc_8^8\, dc_8^1\, d\ell_1\, d\ell_8$$

is a time-dependent measure on the space

$$\mathbb{R}^n \times (\mathbb{R}_0^+)^5$$

, where $\mathbb{R}_0^+$ denotes the set of nonnegative reals and n is the number of spatial dimensions.

More intuitively, $R(t, \mathbf{x}, c_1, c_8^8, c_8^1, \ell_1, \ell_8)$ can be roughly thought of as the number of cells at location $\mathbf{x}$ and time t which have c_1 CG-1A molecules bound to shared counterreceptors on their membranes, c_8^8 CG-8 molecules bound to CG-8 counterreceptor, c_8^1 CG-8 molecules bound to shared counterreceptor, ℓ_1 molecules of CG-1's counterreceptors, and ℓ_8 molecules of CG-8 counterreceptor. [1]

[1] Note on the mathematical notation: Here and elsewhere, the letter ℓ is used to denote various counterreceptor concentrations. The use of this letter refers to the fact that these counterreceptors have also been referred to as "ligands".

For instance the total cell density at a point $\mathbf{x}$ at time t is given by the integral over the various concentrations:

$$\text{cell density at location } \mathbf{x}$$
$$= \int_0^\infty \int_0^\infty \int_0^\infty \int_0^\infty \int_0^\infty R(t, \mathbf{x}, c_1, c_8^8, c_8^1, \ell_1, \ell_8)\, dc_1\, dc_8^8\, dc_8^1\, d\ell_1\, d\ell_8$$

To write expressions as above in a more compact form, we introduce the following notation: we denote the integral over the various concentration as

$$\int \cdots dP = \int_0^\infty \int_0^\infty \int_0^\infty \int_0^\infty \int_0^\infty \ldots\, dc_1\, dc_8^8\, dc_8^1\, d\ell_1\, d\ell_8 \qquad (1)$$

The total concentration of CG-1A at time t and location x (bound to its counterreceptor or freely diffusible) is thus

$$c_1^u(t, \mathbf{x}) + \int c_1 \cdot R(t, \mathbf{x}, c_1, c_8^8, c_8^1, \ell_1, \ell_8)\, dP.$$

The model

In the following, we write down the equations for the cell density R, and the equations for the freely diffusible (unbound) galectins c_1^u and c_8^u.

Equation for the cell density R

The equation for the cell density R takes into account Brownian motion, cell-cell adhesion, binding and unbinding of galectins to counterreceptors, and changes of the counterreceptor concentrations on the cell membranes
(through expression of counterreceptors and detachment of galectins from counterreceptors).
The equation is as follows:

$$\frac{\partial R}{\partial t} = \underbrace{D_R \nabla^2 R}_{\text{cell diffusion}} - \underbrace{\nabla \cdot (R\,\mathbf{K}(\hat{R}))}_{\text{cell-cell adhesion}} \underbrace{-\frac{\partial}{\partial c_1}(\alpha R) - \frac{\partial}{\partial c_8^8}(\beta_8 R) - \frac{\partial}{\partial c_8^1}(\beta_1 R)}_{\text{binding/unbinding of galectins to counterreceptors}} \underbrace{-\frac{\partial}{\partial \ell_1}\left[(\gamma - \alpha - \beta_1)R\right] - \frac{\partial}{\partial \ell_8}\left[(\delta - \beta_8)R\right]}_{\text{change in counterreceptors}} \tag{2}$$

In the above equation, the terms entering the formulas are summarized in Table 2. [2]

[2] A term with a bar over it (for example $\overline{\alpha}_1$) denotes a constant.

$D_R = const$	cell diffusion coefficient
$\alpha = \overline{\alpha}_1 c_1^u \ell_1 - \overline{\alpha}_2 c_1$	change in CG-1A bound to the shared counterrece uptake at rate prop. to $c_1^u \ell_1$; random detachmer
$\beta_8 = \overline{\beta}_{8,1} c_8^u \ell_8 - \overline{\beta}_{8,2} c_8^8$	change in CG-8 bound to its own counterreceptor
$\beta_1 = \overline{\beta}_{1,1} c_8^u \ell_1 - \overline{\beta}_{1,2} c_8^1$	change in CG-8 bound to the shared counterrecept
$\gamma = \overline{\gamma}_1 \frac{c_1}{\overline{c_1}+c_1} - \overline{\gamma}_2 \ell_1$	change in the shared counterreceptor: expression depends on CG-1A concentration; de
$\delta = \overline{\delta}_1 - \overline{\delta_2} \ell_8$	change in CG-8 counterreceptor: expression is **constant** (independent of CG-8 co

Table: Explanation of terms in the cell density equation (2). A term with a bar over it (for example $\overline{\alpha}_1$) denotes a constant.

For example, the term $\gamma - \alpha - \beta_1$ models the rate at which the membrane-bound concentration of the shared counterreceptors which are not bound to either galectin changes:
The change is due to the expression of new counterreceptors by the cells and degradation (leading to the effective rate γ), the binding and unbinding of the counterreceptor to CG-1A (the rate α) and the binding and unbinding of the counterreceptor to CG-8 (the rate β_1).

We assume simple mass action-type dependencies of the rates on the various concentrations.
Note two crucial assumptions that are directly motivated by experimental results:
The rate of expression of CG-1A counterreceptor depends on the concentration c_1 of bound CG-1A (see the formula for γ above); this dependence is modeled with a Michaelis-Menten term.
In contrast, the expression of CG-8 counterreceptor is constant, and thus independent of the concentration of bound CG-8.

Note that equation (2) for the cell density

$$R = R(t, \mathbf{x}, c_1, c_8^8, c_8^1, \ell_1, \ell_8)$$

has formal similarities to the equations for structured populations from the field of mathematical population biology. Finally, the cell-cell adhesion term is formulated based on the approach of [13]. Namely, we have

$$\begin{aligned} &\mathbf{K}(R(t, \mathbf{x}, c_1, c_8^8, c_8^1, \ell_1, \ell_8)) \qquad (3) \\ &= \overline{\alpha}_K \, c_1 \iint_{D_{\rho_0}} \int \tilde{c}_1 \, \sigma(R(t, \mathbf{x}+\mathbf{r}, \tilde{c}_1, \tilde{c}_8^8, \tilde{c}_8^1, \tilde{\ell}_1, \tilde{\ell}_8)) \, d\tilde{P} \, \frac{\mathbf{r}}{|\mathbf{r}|} \, d^n r \end{aligned}$$

Here $\overline{\alpha}_K$ is a constant which represents the strength of the adhesion.
The effective adhesion force on a cell at location $\mathbf{x}$ depends on the product of the concentration of bound CG-1A on the cell and the concentration of bound CG-1A at locations $\mathbf{x} + \mathbf{r}$, where the distance vector $\mathbf{r}$ varies over the $n-$dimensional ball $D_{\rho_0}(\mathbf{x})$ centered at $\mathbf{x}$, where we can consider from one to three spatial dimensions ($n = 1, 2, 3$).
The radius ρ_0 is the "sensing" radius, which is a measure of the characteristic distance for adhesion; cells at distance greater than ρ do not contribute to the adhesion forces.

There are many possible choices for the function $\sigma(R)$, which describes the dependence of the adhesion forces on the cell density.
The simplest choice is a proportionality assumption:

$$\sigma(R) = R \tag{4}$$

In this case, the contribution to the adhesion force from location $\mathbf{x} + \mathbf{r}$ is simply proportional to the concentration of bound CG-1A at that location.
In this model, the cell density can in principle get arbitrarily large.
To avoid this, one can take into account that above a certain density, the effective attractive forces due to cell-cell adhesion are balanced by effectively repellent forces due to volume exclusion; that is, cells cannot be packed into arbitrarily small domains.

This is implemented by a logistic form for the function σ, more precisely [13]:

$$\sigma(R) = \frac{R_m}{\overline{R}(R_m - \overline{R})} R \max\{1 - \frac{1}{R_m} \int R \, dP, 0\}. \qquad (5)$$

Recall that $\int R \, dP$ is the total cell density, and so the above expression involves a volume constraint term. Here R_m is a constant that specifies the maximum cell density for adhesion and $\overline{R} < R_m$ is a characteristic cell density. The proportionality factor above is chosen such that the logistic term (5) has the same value as the linear term (4) if $R(t, \mathbf{x}, c_1, c_8^8, c_8^1, \ell_1, \ell_8)$ is constant in time and spatially homogeneous, more specifically if it has the form $R(t, \mathbf{x}, c_1, c_8^8, c_8^1, \ell_1, \ell_8) = \overline{R} \cdot \phi(c_1, c_8^8, c_8^1, \ell_1, \ell_8)$, where ϕ is some function.

The equation (2) is considered in a spatial domain Ω (one, two or three-dimensional) with normal field $\mathbf{n}(\mathbf{x})$, $\mathbf{x} \in \partial\Omega$. It has the following initial and boundary conditions:

$$\text{initial condition: } R(0, \mathbf{x}, c_1, c_8^8, c_8^1, \ell_1, \ell_8) = R_0(\mathbf{x}, c_1, c_8^8, c_8^1, \ell_1, \ell_8) \tag{6}$$

$$\text{boundary conditions: } \frac{\partial R}{\partial \mathbf{n}}\Big|_{\mathbf{x}} = 0 \text{ for } \mathbf{x} \in \partial\Omega, \tag{7}$$

$$R\big|_{c_1=0} = R\big|_{c_8^8=0} = R\big|_{c_8^1=0} = R\big|_{\ell_1=0} = R\big|_{\ell_8=0} = 0.$$

This means that there are no (diffusive) flux conditions on the boundary of the spatial domain. [3]
We assume that the decay of

$$R(t, \mathbf{x}, c_1, c_8^8, c_8^1, \ell_1, \ell_8)$$

in the non-temporal and non-spatial variables is fast enough so that the integrals $\int R dP, \int c_1 R dP$, etc, are all finite.
The condition that R is zero when one of the concentrations of the various proteins are zero means that the total number of cells is preserved in time, as can be seen from integrating equation (2) with respect to dx and dP.

[3] Alternatively, it is also raesonable to use periodic boundary conditions for the spatial domain in simulations.

Equations for the free galectin concentrations

The equations for the free galectin concentrations c_1^u and c_8^u take into account diffusion, degradation, binding to and detaching from counterreceptors, and secretion by cells. The equations are as follows:

$$\frac{\partial c_1^u}{\partial t} = \underbrace{D_1 \nabla^2 c_1^u}_{\text{diffusion}} \underbrace{+\overline{\nu} \int c_8^8 R \, dP}_{\text{pos. feedback of CG-8 on prod. of CG-1A}} \underbrace{- \int \alpha R \, dP}_{\text{binding of CG-1A to its counterreceptor}} \underbrace{-\overline{\pi}_1 c_1^u}_{\text{degradation}} \tag{8}$$

$$\frac{\partial c_8^u}{\partial t} = \underbrace{D_8 \nabla^2 c_8^u}_{\text{diffusion}} \underbrace{+\overline{\mu} \int c_1 R \, dP}_{\text{pos. feedback of CG-1A on prod. of CG-8}} \underbrace{- \int \beta_1 R \, dP - \int \beta_8 R \, dP}_{\text{binding of CG-8 to counterreceptors}} \tag{9}$$

All terms in the above equations not defined in previous sections are constants.

Again, we have boundary and initial conditions for these equations, which here are

$$\text{initial condition: } c_1^u(0,\mathbf{x}) = c_{1,0}^u(\mathbf{x}), c_8^u(0,\mathbf{x}) = c_{8,0}^u(\mathbf{x}) \quad (10)$$

$$\text{boundary conditions: } \frac{\partial c_1^u}{\partial \mathbf{n}}\Big|_{\mathbf{x}} = \frac{\partial c_8^u}{\partial \mathbf{n}}\Big|_{\mathbf{x}} = 0 \text{ for } \mathbf{x} \in \partial\Omega \quad (11)$$

In [12], a simpler set of equations based on the assumption of fast counterreceptor binding and unbinding.
We define the total concentration of CG-1As counterreceptors (whether unbound or bound to CG-1A or CG-8) to be

$$T_1 = c_1 + c_8^1 + \ell_1. \tag{12}$$

Similarly, the total concentration of CG-8 counterreceptor is

$$T_8 = c_8^8 + \ell_8. \tag{13}$$

After the assumption of "fast galectin binding" and non-dimensionalization, we obtain the following non-dimensional equations:

$$\frac{\partial R}{\partial t} = d_R \nabla^2 R - \nabla \cdot (R\,\mathbf{K}(\hat{R})) \tag{14}$$
$$- \frac{\partial}{\partial T_1}\left(\widetilde{\gamma}(c_1^u, c_8^u, T_1)\, R\right) - \frac{\partial}{\partial T_8}\left(\widetilde{\delta}(c_8^u, T_8)\, R\right)$$
$$\frac{\partial c_1^u}{\partial t} = \nabla^2 c_1^u + \widetilde{\nu} \int_0^\infty \int_0^\infty c_8^8\, R\, dT_1\, dT_8 - c_1^u \tag{15}$$
$$\frac{\partial c_8^u}{\partial t} = \nabla^2 c_8^u + \widetilde{\mu} \int_0^\infty \int_0^\infty c_1\, R\, dT_1\, dT_8 - \widetilde{\pi}_8\, c_8^u. \tag{16}$$

with

$$c_8^8 = c_8^8(t, \mathbf{x}, T_8) = \frac{c_8^u T_8}{1 + c_8^u} \tag{17}$$

$$c_1 = c_1(t, \mathbf{x}, T_1) = \frac{c_1^u T_1}{1 + f c_8^u + c_1^u} \tag{18}$$

$$\widetilde{\gamma}(c_1^u, c_8^u, T_1) = \left(\frac{2c_1^u}{\frac{c_1^u T_1}{c_1^u + f c_8^u + 1} + \widetilde{\bar{c}}_1} - \widetilde{\gamma}_2 \right) \frac{T_1}{c_1^u + f c_8^u + 1} \tag{19}$$

$$\widetilde{\delta}(c_8^u, T_8) = 1 - \widetilde{\delta}_2 \frac{T_8}{1 + c_8^u} \tag{20}$$

$$\mathbf{K}(R(t, \mathbf{x}, T_1, T_8)) = \widetilde{\alpha}_K \, c_1(t, \mathbf{x}, T_1) \int_0^\infty \int_0^\infty \int_{D_{r_0}(\mathbf{x})} c_1(t, \mathbf{s}, \tilde{T}_1) \tag{21}$$

$$\widetilde{\sigma}(R(t, \mathbf{s}, \tilde{T}_1, \tilde{T}_8)) \, \frac{\mathbf{s}}{|\mathbf{s}|} \, ds \, d\tilde{T}_1 \, d\tilde{T}_8$$

Here we can either use a linear or logistic form for $\widetilde{\sigma}$ in the expression for the adhesion flux, as indicated in (4) and (5), respectively; that is

$$\widetilde{\sigma}(R) = R \quad \text{or} \tag{22}$$

$$\tilde{\sigma}(R) = \frac{\widetilde{R}_m}{\widetilde{\overline{R}}(\widetilde{R}_m - \widetilde{\overline{R}})} R \max\left(1 - \frac{1}{\widetilde{R}_m}\int_0^\infty \int_0^\infty R\, dT_1\, dT_8, 0\right) \tag{23}$$

$$\text{with } \widetilde{R}_m = \frac{R_m}{\hat{R}}, \quad \widetilde{\overline{R}} = \frac{\overline{R}}{\hat{R}}.$$

This system of equations was analyzed numerically in Glimm, Bhat, Newman [12].

There are many analytic questions open: existence and uniqueness of solutions and positivity.

Preliminary test

Consider the most important part of the main problem

$$\frac{\partial R}{\partial t} = d_R \nabla^2 R - \nabla \cdot (R\,K) - \frac{\partial}{\partial T_1}(\gamma\, R) - \frac{\partial}{\partial T_8}(\delta\, R)$$

$$\frac{\partial c_1^u}{\partial t} = \nabla^2 c_1^u + g^u - c_1^u$$

$$\frac{\partial c_8^u}{\partial t} = \nabla^2 c_8^u + h^u - \widetilde{\pi}_8\, c_8^u.$$

with

$$R(0, \mathbf{x}, T_1, T_8) = R_0(\mathbf{x}, T_1, T_8)$$

$$\frac{\partial R}{\partial \mathbf{n}}\Big|_{\mathbf{x}} = 0 \quad R\big|_{c_1=0} = R\big|_{c_8^8=0} = R\big|_{c_8^1=0} = R\big|_{\ell_1=0} = R\big|_{\ell_8=0} = 0.$$

$$c_1^u(0, \mathbf{x}) = c_{1,0}^u(\mathbf{x}), c_8^u(0, \mathbf{x}) = c_{8,0}^u(\mathbf{x}), \quad \frac{\partial c_1^u}{\partial \mathbf{n}}\Big|_{\mathbf{x}} = \frac{\partial c_8^u}{\partial \mathbf{n}}\Big|_{\mathbf{x}} = 0$$

If γ, δ are bounded, positive, then we have

$$+ \to + \text{ and } L^1 \to L^1.$$

Morover, "natural" FDM's are stable.

$$\frac{R(t+\Delta t,...)-R}{\Delta t} = d_R \nabla^2_{\Delta x} R - \nabla' \cdot (R\,K) - \frac{\Delta_-(\gamma\,R)}{\Delta T_1} - \frac{\Delta_-(\delta\,R)}{\Delta T_8}$$

$$\frac{c_1^u(t+\Delta t,...)-c_1^u}{\Delta t} = \nabla^2_{\Delta x} c_1^u + g^u - c_1^u$$

$$\frac{c_8^u(t+\Delta t,...)-c_8^u}{\Delta t} = \nabla^2_{\Delta x} c_8^u + h^u - \widetilde{\pi}_8\, c_8^u.$$


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