Phenotype divergence and cooperation in isogenic multicellularity and in cancer

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Plan of the talk

1. Isogenic multicellularity, cancer and the atavistic theory of cancer
2. Phenotype divergence and cooperation in multicellularity and in cancer
3. Modelling phenotype divergence with reaction-diffusion-advection equations
4. Modelling cooperation with the prisoner's dilemma and with PDEs
5. Modelling combined phenotype divergence and cooperation

Physiological isogenic multicellularity and cancer

- Multicellularity is a mandatory framework to be considered when trying to understand, explain and fight cancer, a disease of multicellular animals only. Multicellularity is physiologically a daring construction from the zygote, needing *strict control of cohesion* of its cell constituents. Such control is lost in cancer.

- *Phenotype cell plasticity*, potential of dynamic and reversible phenotype change in cells according to a developmental program or by adaptation to a changing microenvironment, is a mandatory, but transient, *of epigenetic nature*, cellular trait in development, which cannot be kept in cells of an achieved multicellular organism, lest the organism be in permanent *danger of losing its cohesion*. Cellular plasticity is central in cancer, due to loss of control on differentiations.

- Cancer is indeed primarily loss of the normal local *epigenetic* control mechanism on differentiations, work of a coherent set of *gene regulatory networks* involved in the species *body plan*, i.e., the deterministic 'program of making an animal', in a given species, transmitted from the initial egg to all cells in the making, and maintaining its cohesion. Secondarily, cancer is loss of control on proliferations.

- Physiological cell differentiation makes sense within cell lineages, starting from the zygote and following the *body plan*, with the purpose to develop a) *cell specialisations* and b) *compatibility and cooperativity* between specialised cells (*division of labour*). It opposes and restricts cell plasticity, progressively lost in successive physiological differentiations, until terminally differentiated cell types.

The two settings: Darwinian evolution and development, or: *a story of how ontogeny recapitulates phylogeny*

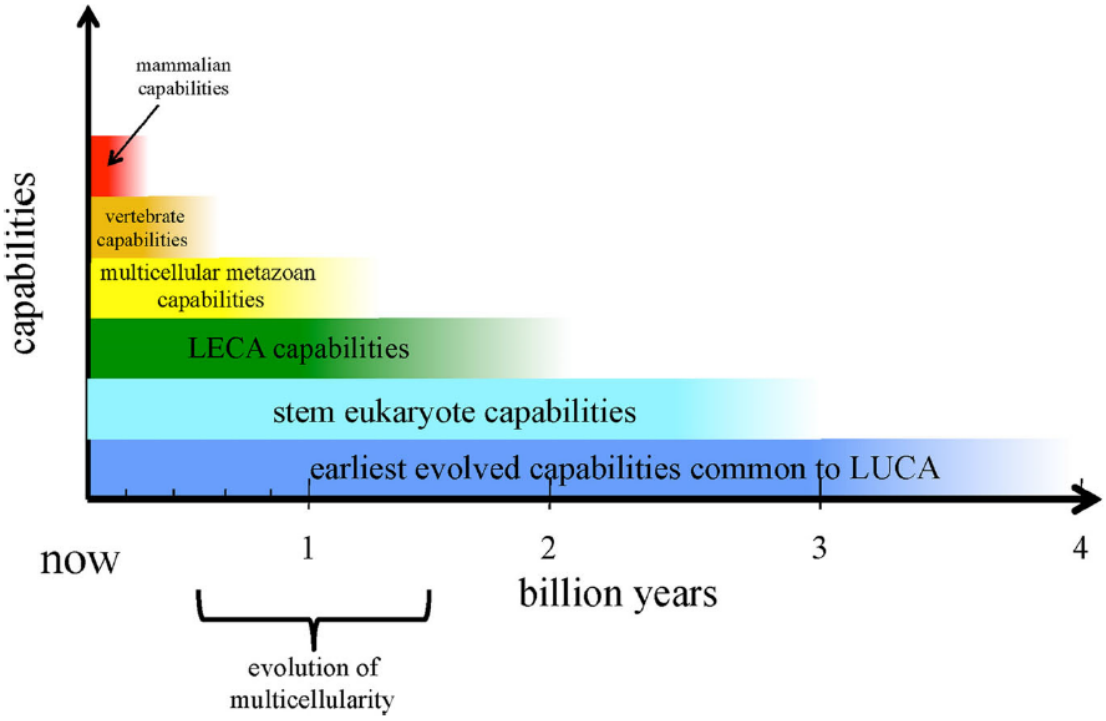
- In the billion-year perspective of Darwinian evolution of animal species, phenotype divergence was likely imposed by environmental constraints, as different ways to optimally solve existential problems due to new conditions of living. When adaptation gave way to such divergent specialisations in the same changing conditions for one given species, the divergent choices made were likely random, firstly reversible, later irreversible, due to fixation by mutations.

- On the contrary, in the process of multicellular development from the initial egg in a given animal species, phenotype divergence and resulting successful cooperations are completely deterministic, written in the program of the body plan of each species. The body plan, borne in each cell of the organism is the evolutionary unit with which Darwinian evolution of species is written. This is how physiologically (deterministic) '*ontogeny recapitulates* (random) *phylogeny*'.

- Cancer alters the maintenance of the anatomically and physiologically unfolded body plan by the ensemble of gene regulatory networks that make its cohesion. However, tumour cells keep in their genome facilities, written in the program of their body plan, to develop specialisations (possibly *with bet hedging*) and cooperations inherited from their evolutionary past, that can easily be recruited to face environmental changes, as they have acquired uncontrolled plasticity.

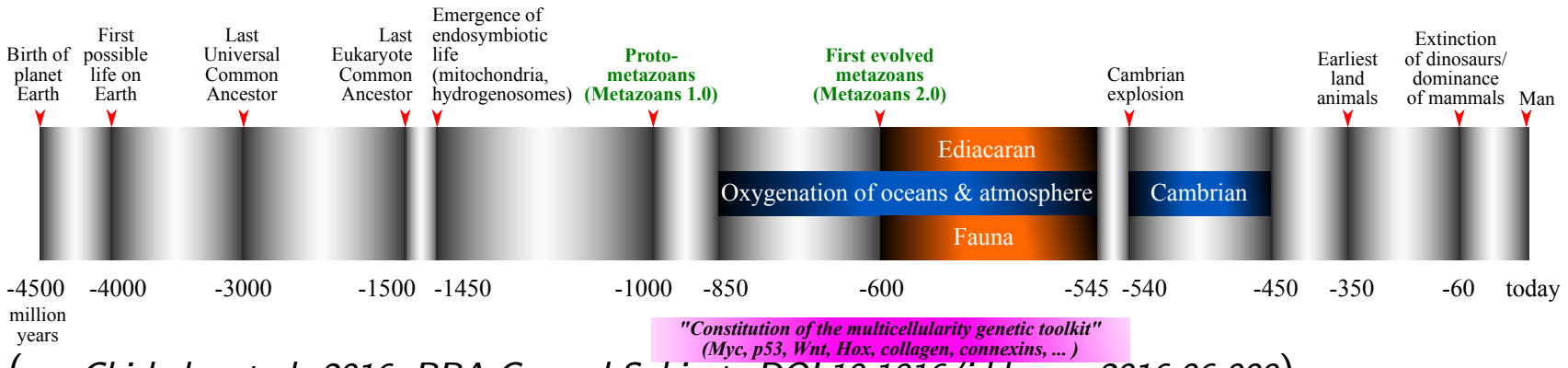
A billion-year evolutionary framework: *the atavistic theory of cancer* provides a vision of cancer as a regression towards a coarse, unachieved, incoherent form of multicellularity

"Nothing in biology makes sense except in the light of evolution" (Th. Dobzhansky, 1973)



"Cancer: more archeoplasm than neoplasm" (Mark Vincent, 2011) More references: Israel JTB 1996, Davies & Lineweaver Phys Biol 2011, Vincent Bioessays 2011, Lineweaver, Davies & Vincent Bioessays 2014, Lineweaver et al. 2020, 2021, Trigos et al. PNAS 2017, BJC 2018, eLife 2019, bioRxiv 2023.

A billion-year evolutionary framework: *the atavistic theory of cancer* provides a vision of cancer as a regression towards a coarse, unachieved, incoherent form of multicellularity



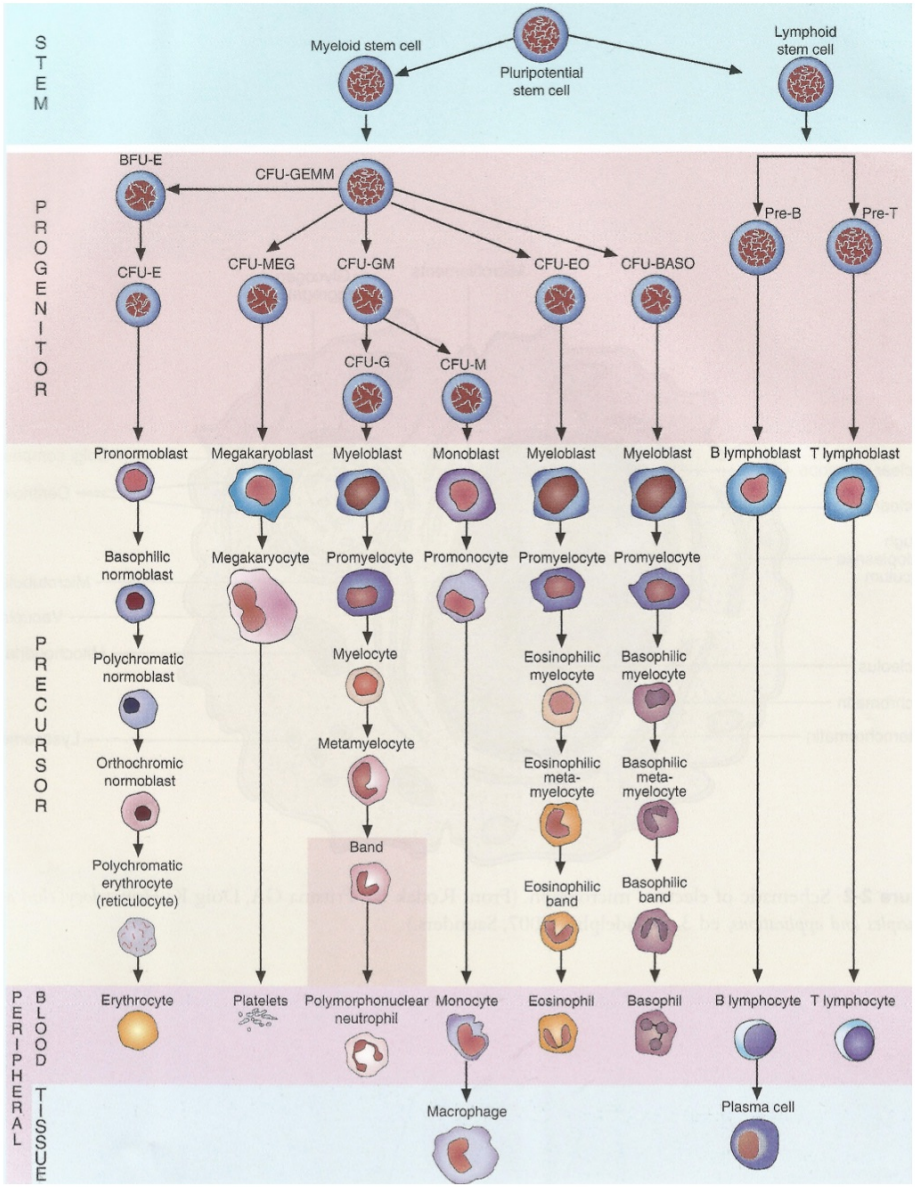
(*see Chisholm et al. 2016, BBA General Subjects DOI:10.1016/j.bbagen.2016.06.009*)

- The genes that have appeared in the development of multicellularity are those that are altered in cancer: phylostratigraphic analyses by Domazet-Lošo & Tautz 2010; multicellularity vs. unicellularity gene investigations by Trigos *et al.* 2017, 2018, 2019, 2023 show overexpression of unicellularity genes and underexpression of multicellularity genes in cancer.
- Evolution order: 1) proliferation + contact inhibition to 2) cell differentiation + division of work, and to 3) achieved *epigenetic control* on differentiation and proliferation? (reverse mutation order in AML, *Hirsch Nature Comm. 2016*).
- Attacking cancer on proliferation is precisely attacking its robustness. It is better to attack its weaknesses: absence of protecting immune system in tumours.

The *body plan* unfolds in normal development according to branchings by successive phenotype divergences in all cell lineages from the egg to terminally differentiated cell types

A personal metaphor: the wickerwork basket. A fibre bundle (base, the *body plan* in the zygote, i.e., the initial egg); fibres, the cell differentiation trees; at the rim of tips, terminally differentiated cells). Intertwining the trees that stem from the body plan are between-fibre connections (e.g., intercellular metabolic gene regulatory networks of epigenetic nature) that *control the coherence (in compatibility/cooperativity) of differentiations*, making their coherent ensemble a *cohesion watch*, part of the design of the *body plan*, which is primarily disrupted in cancer, impairing differentiations.

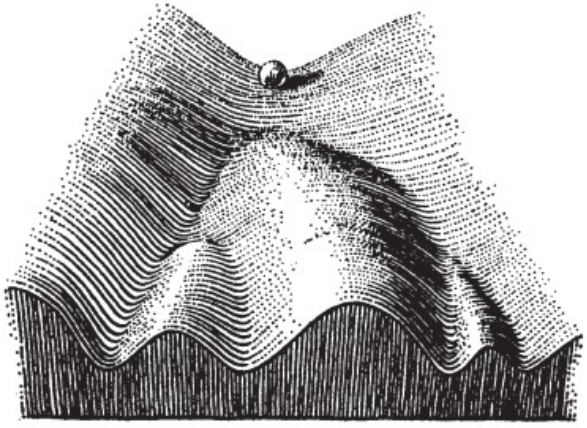
The best known case in development: haematopoiesis



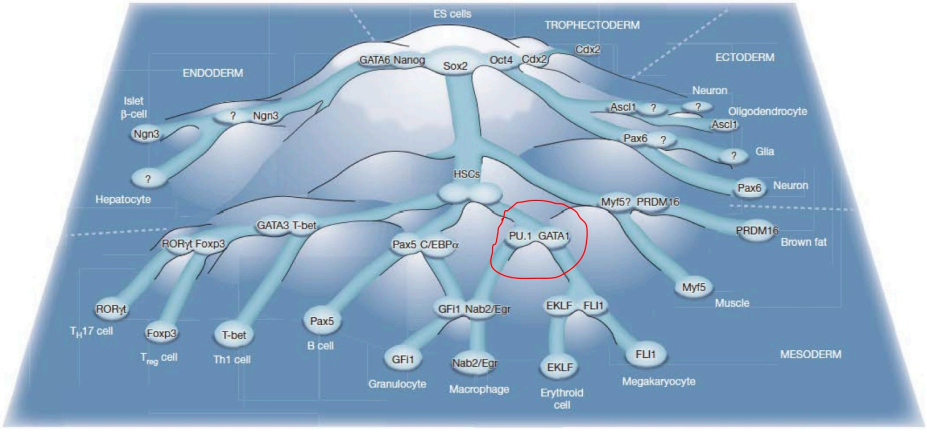
Haematopoiesis, after Carr & Rodack, *Clinical hematology atlas*, 2012.

Succession of phenotype divergences, i.e., cell specialisations, followed by successive within-lineage differentiations, from the pluripotent haematopoietic stem cell (top) until the terminally differentiated formed cells in tissues, mainly blood (bottom).

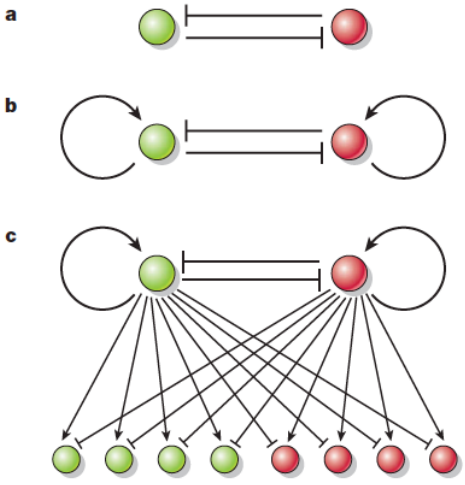
Milestones to reconstruct the global differentiation landscape

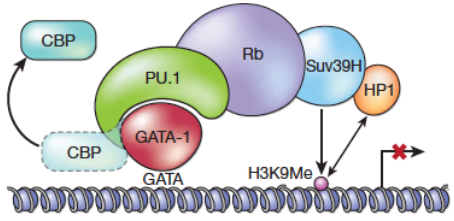


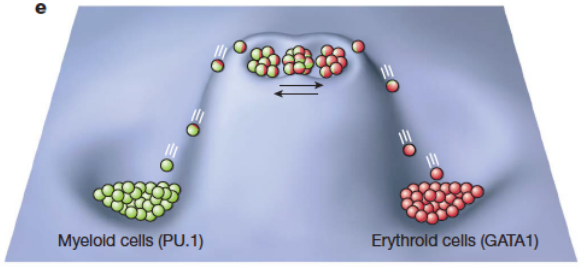
[A classic metaphor: the Waddington landscape]



Stem cell fate: modern version by Tariq Enver *(ASH meeting 2011)*







Zoom on the PU.1/GATA1 node (for equations and bifurcations, see Huang, Guo, May & Enver *Devel Biol 2007*)

Cell population model with trade-offs between phenotypes to represent divergences in the unfolding of the body plan

Or: *bet hedging* as a 'tumour strategy' to diversify its phenotypes in response to deadly stress (cytotoxic drugs) Let $D = \Omega \times [0,1]$, where $\Omega := \{C(x,y) \leqslant K\}$ (a constraint between traits x and y). The evolution of a plastic cell population $n(z,t)$ structured in a 3D phenotype $z = (x,y,\theta)$, where, e.g., x=viability, y=fecundity, and θ=plasticity, is given by

$$\partial_t n + \nabla \cdot \Big(Vn - A(\theta)\nabla n \Big) = (r(z) - d(z)\rho(t))n,$$

with $\left(\text{Vn-A}(\theta)\nabla n\right) \cdot \mathbf{n} = 0$ for all $z \in \partial D$; $n(0,z) = n_0(z)$ for all $z \in D$, where $\Omega = \{(x,y) \in [0,1]^2 : (x-1)^2 + (y-1)^2 > 1\}$, and the diffusion matrix $A(\theta) = \begin{pmatrix} a_{11}(\theta) & 0 & 0 \\ 0 & a_{22}(\theta) & 0 \\ 0 & 0 & a_{33} \end{pmatrix}$, with a_{11} and a_{22} non-decreasing functions of θ, influences the speed at which non-genetic epimutations occur, otherwise said, it is a representation of how the internal plasticity trait θ affects the non-genetic instability of traits x and y, by tuning the diffusion term $\nabla.\{A(\theta)\nabla n\}$; the advection term

$$\nabla.\{V(t,z)n\} = \nabla.\{(V_1(t,z), V_2(t,z), V_3(t,z))n\}$$

represents the cellular stress exerted by external evolutionary pressure on the population, i.e., by changes in the environment; and $\rho(t) = \int_D n(t,z)dz$ is the total mass of individual cells in the population at time t.

(FEA, JA Carrillo, JC, J. Math. Biol. 2022)

Phenotype divergence: numerics

The existence and uniqueness of solutions may be obtained *in finite horizon* by numerical methods showing convergence of the algorithms used to discretise the model. Illustrations may be obtained with instances of the functions used in the equations. For instance, to obtain phenotypic divergence (which we take as the basis of both bet hedging in cancer and of emergence of multicellularity in evolution), we consider over the domain $D = \Omega \times [0,1]$ an initial density given by the expression

$$n_0(z) = a 1_{\{f(z)<1\}} e^{-\frac{1}{1-f(z)}},$$

with $f(z) = \frac{\|z-z_0\|^2}{(0.025)^2}$, where $z_0 = (0.25, 0.25, 0.5)$ and $\|\cdot\|$ is the euclidean norm. We choose the value of a in such a way that $\rho_0 = \int_D n_0(z) = 1$.
We set the growth rate and the death rate as

$$r(x,y,\theta) = 1_{\{y>x\}} e^{-(0.1-x)^2-(0.9-y)^2} + 1_{\{x\geqslant y\}} e^{-(0.1-y)^2-(0.9-x)^2},$$
$$d(x,y,\theta) = \frac{1}{2}.$$

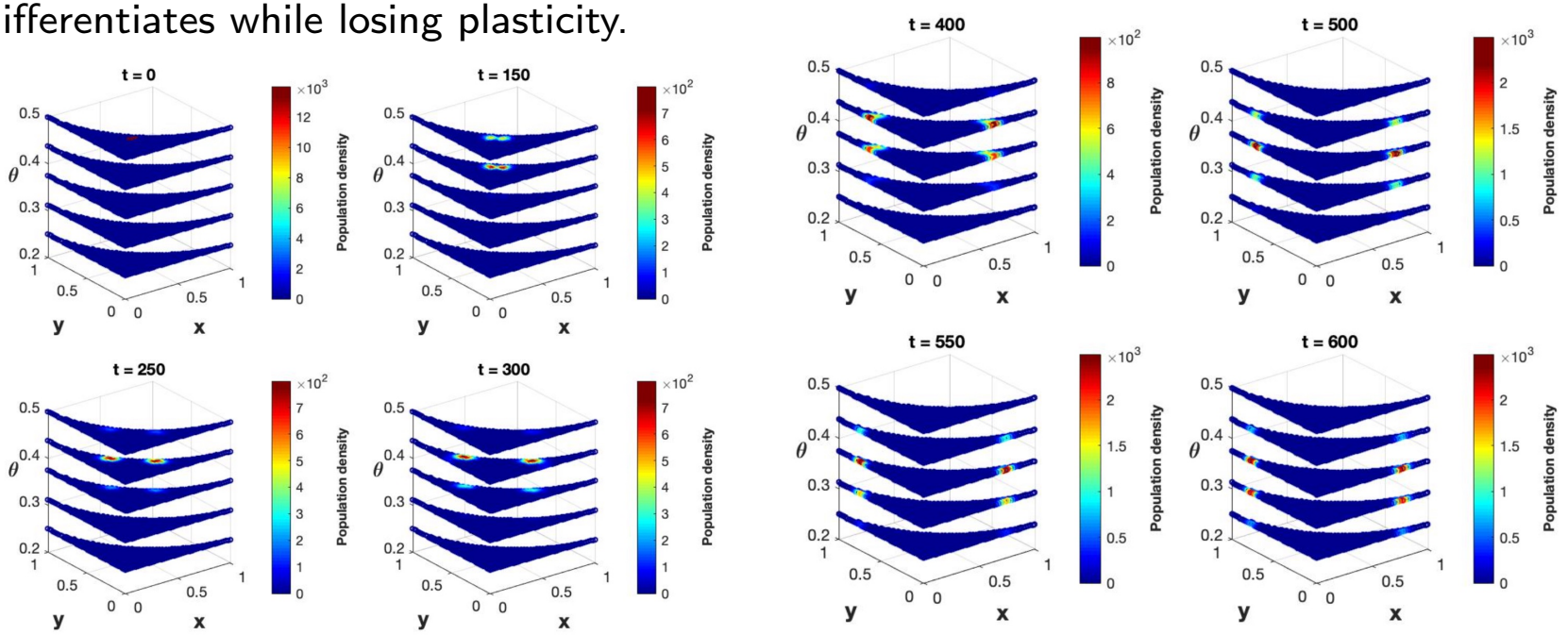
We choose the diffusion matrix

$$A(\theta) = \begin{pmatrix} (\theta+1)10^{-6} & 0 & 0 \\ 0 & (\theta+1)10^{-6} & 0 \\ 0 & 0 & 10^{-6} \end{pmatrix}, \textit{and}$$

the advection term $V(t,z) = 10^{-3}(-y,-x,-(x+y))$ or $10^{-3}\theta(-y,-x,-(x+y))$.

Phenotypic divergence: illustration (first stages)

The "push" towards specialisation imposed by V is negatively proportional to the current set of traits (individuals with traits (x, y) are specialising with a rate proportional to $(-y, -x)$). We see on the illustration below that initially the population is concentrated around the phenotype $z_0 = (0.25, 0.25, 0.5)$, and gradually differentiates while losing plasticity.



Initial stages of the population density for different values of θ: the differentiation process starts. At around $t = 250$ (bottom left) most of the population has already concentrated around the plasticity level $\theta = 0.4375$ and around $t = 300$ (bottom right) we observe that the migration towards a less plastic state continues. Around $t = 500$ most of the population has reached $\theta = 0.375$ and at subsequent times the migration continues.

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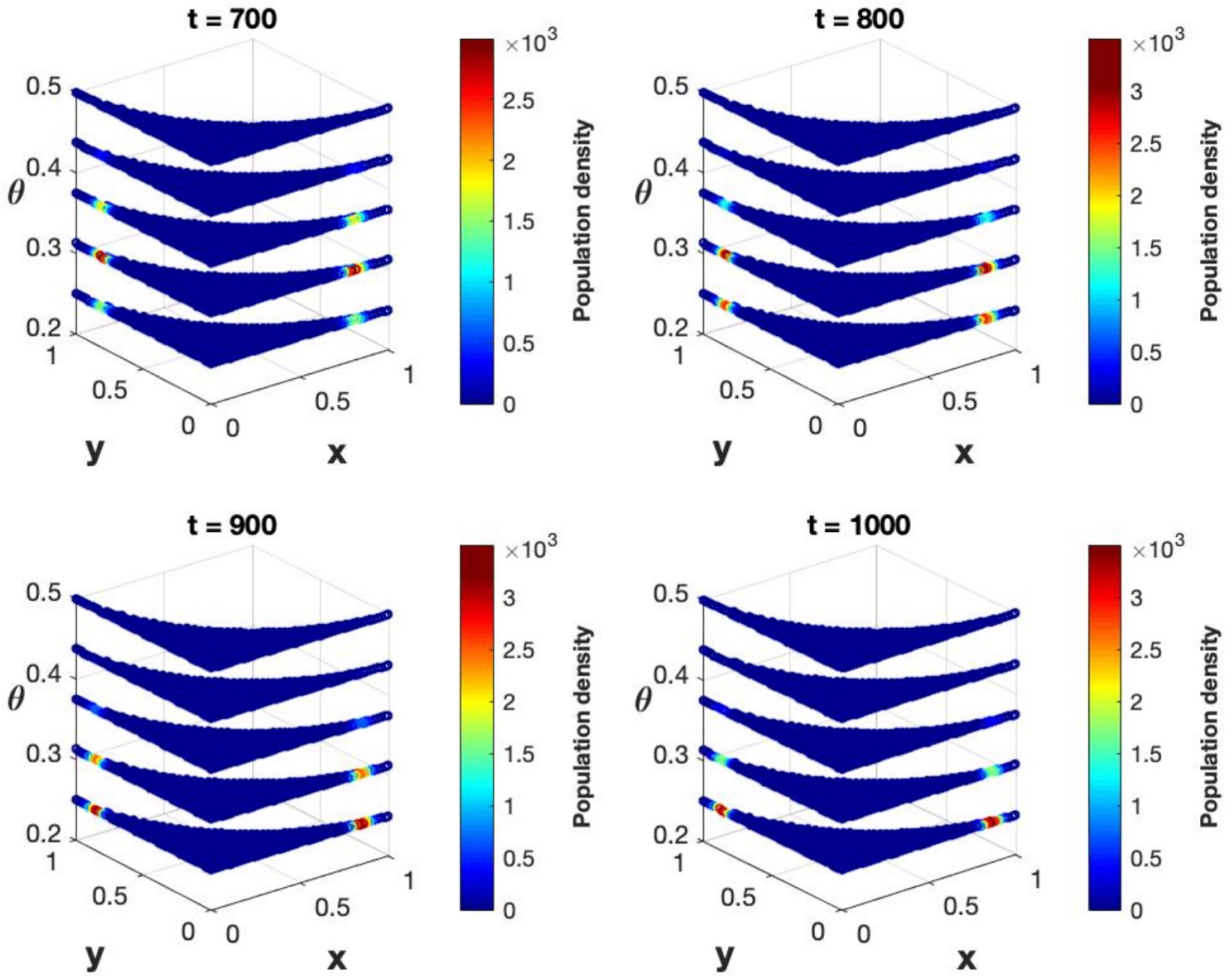
Phenotypic divergence: illustration (final stages)



Final stages of the population density for different values of θ (end): around $t = 900$ (bottom left) the differentiation process is over and most of the population has reached the plasticity level $\theta = 0.25$. At time $t = 1000$ (bottom right) we observe that the population concentrated around any other level of plasticity is almost extinct, and only the one around $\theta = 0.25$ survives.

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Cooperation: the prisoner's dilemma paradigm

An initial intention for cooperation and the existence of reciprocity are crucial for the evolution of cooperation (Axelrod & Hamilton, 1981).
Consider two players involved in the repeated prisoner's dilemma game. Player A will initially cooperate with probability $p_0 > 0$ while player B will do so with probability $q_0 > 0$ (initial intention for cooperation).
Both players will modify their probabilities of cooperation at turn $k+1$ by following the rule:

$$p_{k+1} = \begin{cases} p_k + \varepsilon_{11}(1 - p_k), & \text{if player B cooperated in turn k,} \\ p_k(1 - \varepsilon_{12}), & \text{if not,} \end{cases}$$

and

$$q_{k+1} = \begin{cases} q_k + \varepsilon_{21}(1 - q_k), & \text{if player A cooperated in turn k,} \\ q_k(1 - \varepsilon_{22}), & \text{if not,} \end{cases}$$

where $0 < \varepsilon_{ij} < 1$ for $i, j \in \{1, 2\}$ are reciprocity coefficients (existence of reciprocity).

Cooperation: the prisoner's dilemma paradigm (stable steady states)

Consider a couple (p_0, q_0) and the value $e = \varepsilon_{11}\varepsilon_{21} - \varepsilon_{12}\varepsilon_{22}$.

i) If $e < 0$, then the only possible steady states are $(0,0)$ (stable) and $(1,1)$ (unstable).

ii) If $e > 0$, then the only possible steady states are $(0,0)$ (unstable) and $(1,1)$ (stable).

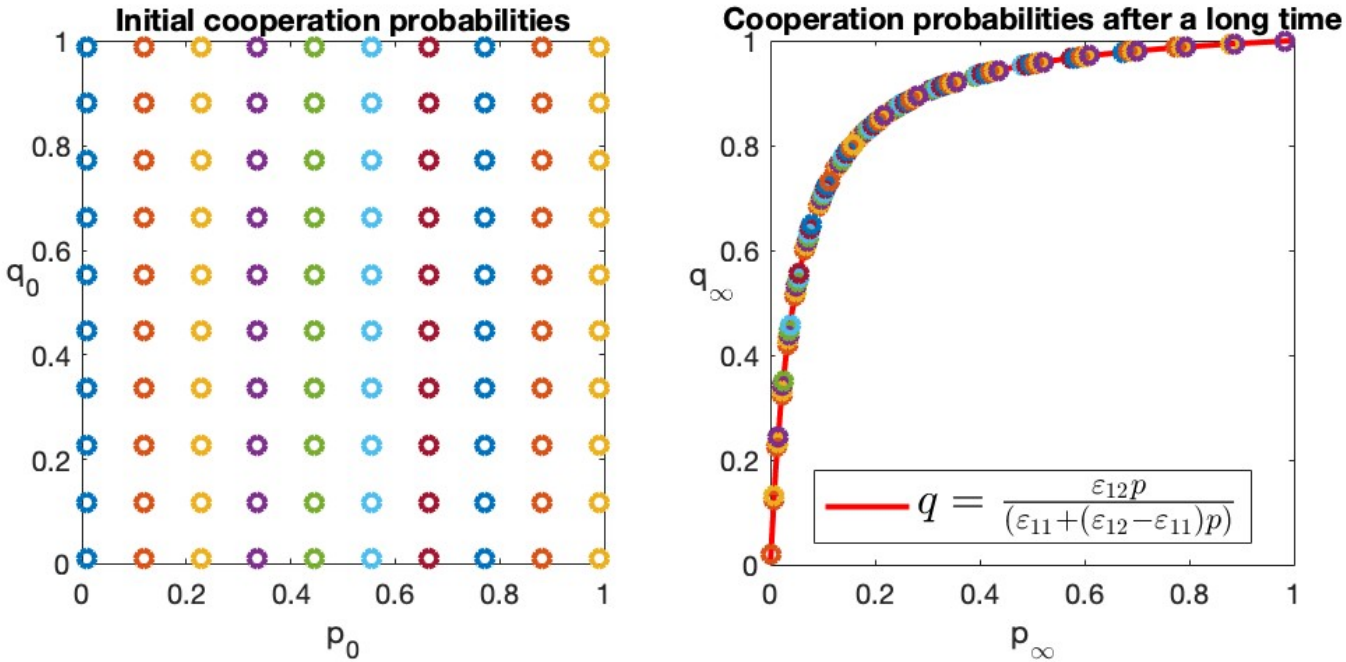
iii) If $e = 0$ then the steady state is the unique solution of

$$\varepsilon_{22}p_0 + \varepsilon_{11}q_0 = \varepsilon_{22}p^* + \varepsilon_{11}q^*,$$

$$q^* = \frac{\varepsilon_{12}p^*}{\varepsilon_{11} + (\varepsilon_{12} - \varepsilon_{11})p^*},$$

and it is a stable steady state.

Cooperation: the prisoner's dilemma paradigm (illustration)



Left panel: Several initial configurations of cooperation probabilities. Right panel: Limiting values of the sequences (p_k, q_k) associated to initial values showcased on the previous figure.

Cooperation between subpopulations: a first PDE model

Let $p \in [0,1]$ be a continuous structure variable representing the probability of cooperation. Consider two populations A and B, each one composed by individuals with different probabilities of interspecific cooperation. Let $n_A(t,p)$ and $n_B(t,p)$ be their respective population densities.
For the two total population masses at time t :

$$\rho_A(t) := \int_0^1 n_A(t,p)dp \text{ and } \rho_B(t) := \int_0^1 n_B(t,p)dp,$$

Mean cooperation probabilities :

$$\tilde{p}_A(t) := \frac{\int_0^1 p n_A(t,p)dp}{\rho_A(t)} \text{ and } \tilde{p}_B(t) := \frac{\int_0^1 p n_B(t,p)dp}{\rho_B(t)}.$$

Global expected gains :

$$\begin{aligned} E_A(t) :=& (b-c)\tilde{p}_A(t)\tilde{p}_B(t) + b(1-\tilde{p}_A(t))\tilde{p}_B(t) - c\tilde{p}_A(t)(1-\tilde{p}_B(t)) \\ =& b\tilde{p}_B(t) - c\tilde{p}_A(t), \\ E_B(t) :=& b\tilde{p}_A(t) - c\tilde{p}_B(t). \end{aligned}$$

where b and c are the benefit and cost, respectively, of cooperation in the prisoner's dilemma setting.

Cooperation between subpopulations: a first PDE model

Both population densities evolve according to the PDE system

$$\begin{cases} \partial_t n_A(t,p) + \varepsilon_A \partial_p \left((\tilde{p}_B(t) - p) n_A(t,p) \right) = & g_A(p, E_A(t)) n_A(t,p), \\ \partial_t n_B(t,p) + \varepsilon_B \partial_p \left((\tilde{p}_A(t) - p) n_B(t,p) \right) = & g_B(p, E_B(t)) n_B(t,p), \\ n_A(0,p) = n_A^0(p), \quad n_B(0,p) = n_B^0(p), \end{cases}$$

where ε_A and ε_B are reciprocity coefficients and g_A, g_B are continuous and increasing functions of E_A and E_B respectively.
For example :

$$\begin{aligned} g_A(p, E_A(t)) &:= r_A(p) + \gamma_A(p) E_A(t) = r_A(p) + \gamma_A(p)(b\tilde{p}_B(t) - c\tilde{p}_A(t)), \\ g_B(p, E_B(t)) &:= r_B(p) + \gamma_B(p) E_B(t) = r_B(p) + \gamma_B(p)(b\tilde{p}_A(t) - c\tilde{p}_B(t)), \end{aligned}$$

Cooperation between subpopulations: a first PDE model

Consider $\varepsilon_A = \varepsilon_B = 0$, $\gamma_A(p) \equiv \gamma_A \geqslant 0$ and $\gamma_B(p) \equiv \gamma_B \geqslant 0$.
Suppose that $r_A(p)$, $r_B(p)$, $n_A^0(p)$ and $n_B^0(p)$ belong to $C([0,1])$, and

$$\arg \max_{p \in supp\ n_A^0} r_A(p) = \{p_A^*\} \text{ and } \arg \max_{p \in supp\ n_B^0} r_B(p) = \{p_B^*\}.$$

Then we can show that

i) If $r_A(p_A^*) + \gamma_A(bp_B^* - cp_A^*) < 0$, population A will go extinct.

ii) If $r_A(p_A^*) + \gamma_A(bp_B^* - cp_A^*) > 0$, there exists and interval I satisfying $p_A^* \in I \subset [0,1]$ such that population A will blow up for all $p \in I$.

iii) The same is true for population B, depending on the sign of $r_B(p_B^*) + \gamma_B(bp_A^* - cp_B^*)$.

This result serves solely to illustrate the, sometimes dramatic, effect of cooperation over the dynamics of two populations. However, this model only accounts for the effect of cooperation and it does so independently of the population sizes. Two flaws to overcome if more realistic scenarios are to be represented. These can be achieved, for example, by integration with the phenotypic divergence model and considering the parameters b, c, γ_A and γ_B as functions of ρ_A and ρ_B,

Combining specialisation with cooperation in a PDE model?

... Work underway...

- At this step of modelling, we can combine the two PDE models by identifying $n_A(p)$ with $n(0.1, 0.9, p)$ and $n_B(p)$ with $n(0.9, 0.1, p)$ in the phenotype divergence model presented above, identifying plasticity θ with a probability of cooperation. This would mean that an initially undivided cell population firstly diverges in phenotypes, i.e., specialises, and that only secondly (and independently of phenotype divergence) cooperation may emerge.

- However, admitting that cooperation with division of work is what makes the meaning of developing multicellularity in the deterministic body plan, one may put the problem the other way round: division of work is a way to optimise a global fitness (to be defined), relying on simultaneous specialisation and cooperation as two complementary populational settings, to be properly defined.

- It thus remains for us to define - and solve - an optimisation problem of global fitness, that should lead from a phenotypically homogeneous cell population to a split one, consisting of two subpopulations, specialised and cooperating, doing better in fitness that the initial one. Which is our present goal in modelling physiological multicellularity, before considering the case of cheating cancer cells.

... Work underway...

In conclusion, what possible consequences for cancer?

- A long-term perspective: modelling the body plan with its cohesion mechanisms (gene regulatory networks, to be searched for) that ensure the global and local (tissue) stability of the animal multicellular organism which is the result of its unfolding in embryogenesis, and also ensure a permanent maintenance of its cohesion in the constituted animal.

- In a more local way, at the scale of each tissue rather than at the scale of the organism, there is a need to explore and represent mechanistically the gene regulatory networks that are at work in tissues, controlling local cell differentiations and proliferation, that are impaired in all cancers.

- Such genetic mechanisms have been explored by evolutionary biologists (W. Müller; E. Davidson and colleagues, exploring genes in the body plan) from the point of view of Darwinian evolution, but not, to the best of our knowledge, from the point of view of their alterations in cancer.

- When alteration of differentiation control is located within the cancer cell itself, in particular by chromosome translocations (such as in Acute Promyelocytic Leukaemia or Chronic Myelogenous Leukaemia), spectacular successes have been obtained (ATRA and AsO_2 for APL, Imatinib and other molecules for CML). But can we change the focus from the cancer cell to the cancer tissue?

... and cancer therapeutics?

- Plasticity in tumour cells leads them to deploy defence mechanisms stored in their genome during their evolutionary past, resulting in various drug resistance mechanisms, in de-differentiation and plastic bet hedging with different cell phenotypes adapted to different insults, or to cooperation between different tumour clones to survive, or in transdifferentiation (such as EMT in metastasis).

- Drug-induced drug resistance in cancer, in particular, is initially at least, a reversible phenomenon, likely of epigenetic nature, that can be thwarted in combined treatments minimising by *optimal control methods* drug exposure, as proposed in *[Pouchol, JC, Lorz & Trélat, J Maths Pures Appl. 2018]*.

- Using epigenetic drugs to thwart EMT, or drugs susceptible to alter cooperation between subclones in tumours are other tracks to explore and further develop.

- Exploring and re-establishing, whenever possible, local tissue control mechanisms may be of no avail when letal driver mutations have occurred in the genome of cancer cells, leading them to complete escape from external control.

- Which implies in this case that the axes explored in the present work resort more to cancer prevention than to treatment of the constituted disease. Nevertheless, immunotherapies, as modelled in *[Kaid, Pouchol & JC MMNP, to appear 2023]*, not involving drug resistances, can then be used. However, while drug-induced resistance is then in principle excluded, toxicity issues may limit their application.

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