Multiple States in Cancer Cell Population and Transplantation Experiment Inference

Yue Wang

Institut des Hautes Études Scientifiques (IHÉS), France

Mar. 10, 2021

Outline

- Two mathematical biology projects: Given certain biological data, what biology can we learn, and what mathematics can we work on?
- Cancer cell population: When there are enough data, what patterns can we reveal? Explain biological phenomena with ODEs and stochastic processes.
- Tissue transplantation: When there are not enough data, can we infer unknown data? Turn experimental design into combinatorial problems.

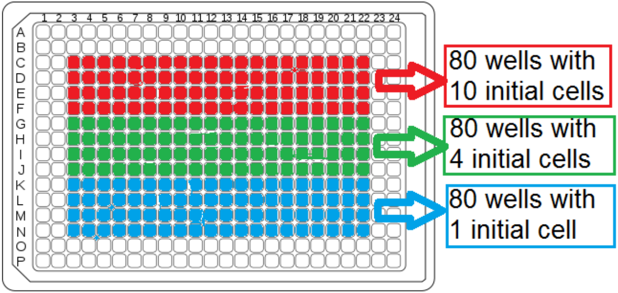
Multiple States in Cancer Cell Population

Section I: Outline

- Cancer cell population is often thought to be homogeneous.
- Analyze experimental data to reveal the existence of multiple cell states.
- Theoretical explanations of related new phenomena.

Section I.1: Experiments

- Cultivate HL60 leukemia cells *in vitro*.



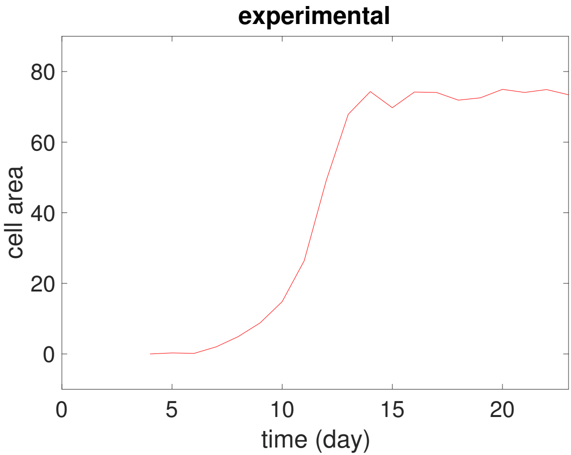
80 wells with 10 initial cells

80 wells with 4 initial cells

80 wells with 1 initial cell

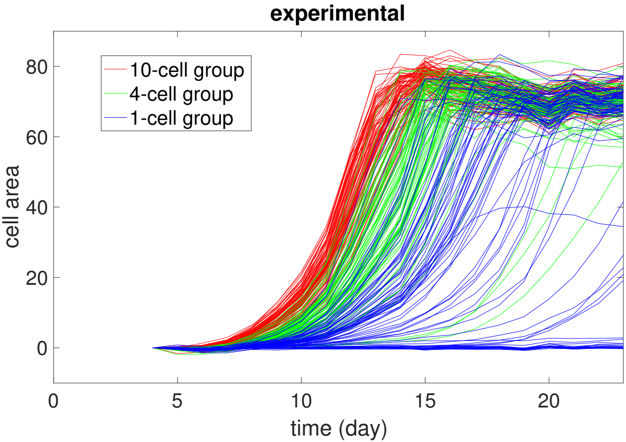
- Initial cells are sampled randomly from a large population.
- For each well, the cell area (proportional to cell number) is measured everyday.

Section I.1: Growth curves



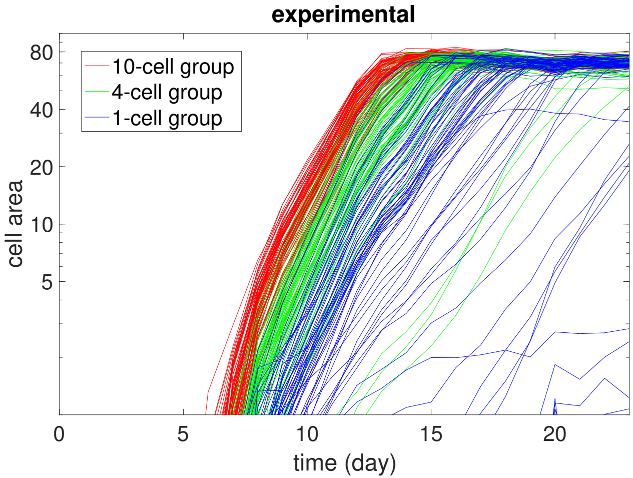
Growth curve of one well, describing how the population changes along time. In general, the population grows exponentially until saturation.

Section I.1: Growth curves



Each growth curve corresponds to one well. Red: 10 initial cells; green: 4 initial cells; blue: 1 initial cell.

Section I.1: Growth curves



Growth curves with *y*-axis in log scale. Red: 10 initial cells; green: 4 initial cells; blue: 1 initial cell.

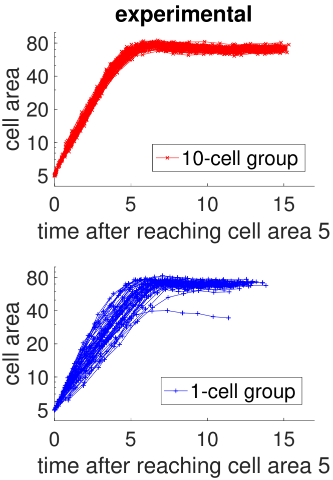
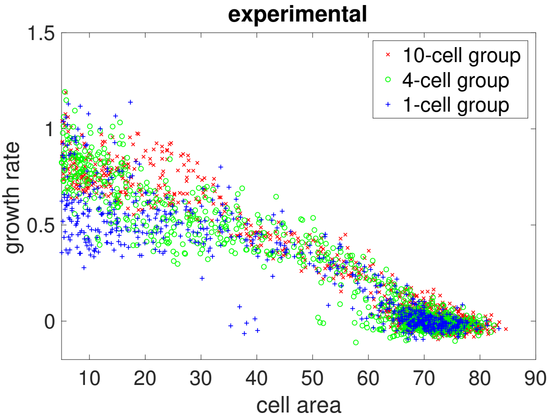


Figure: Translated population curves, starting from cell area 5. *Y*-axis is in log scale. Some 1-cell-wells never reach cell area 5, thus are not shown.

Section I.1: Growth rates

For one well, denote the population at day n as c_n, and the population at day $n+1$ as c_{n+1}. Then the growth rate is $g_n = (c_{n+1} - c_n)/c_n$. For each well in each day, draw the growth rate g_n versus the population c_n. The point cloud near $(75, 0)$ corresponds to saturated wells.

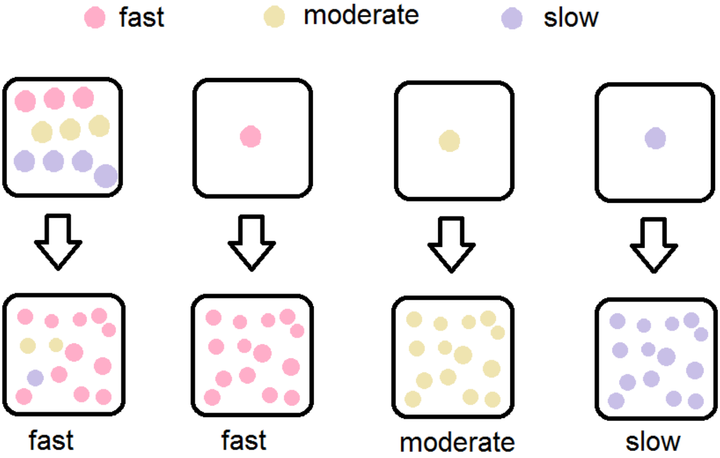


Section I.1: Experimental phenomena

- After reaching the same population, all 10-cell-wells grow fast; some 1-cell-wells grow much slower.
- Some 1-cell-wells keep at low population levels for a long time.
- When a 1-cell-well grows to have 10 cells, it is different from a 10-cell-well.
- Cells cannot be homogeneous.

Section I.1: Analysis

We assume that there are at least three cell states with different growth rates: fast, moderate, and slow.



Section I.1: Model

- Build a multi-type branching process model.
- Initial cells have three possible states, determining the growth rate. Growth rate is inheritable, and decreases as total population increases.
- For each time period, each cell has a probability to divide, and a probability to die.
- In simulation, this model can reproduce most experimental phenomena within a wide range of parameters.

Section I.1: Model

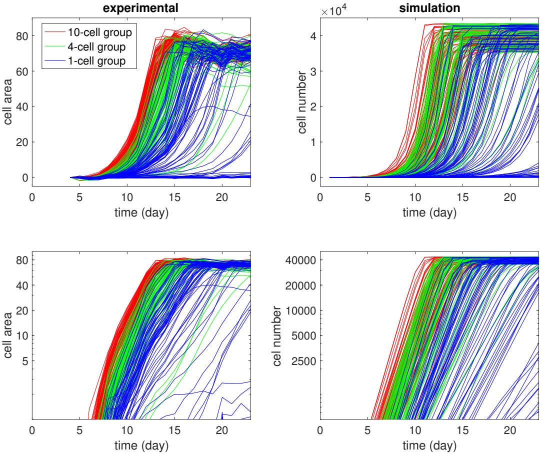


Figure: Population growth curves.

Section I.1: Model

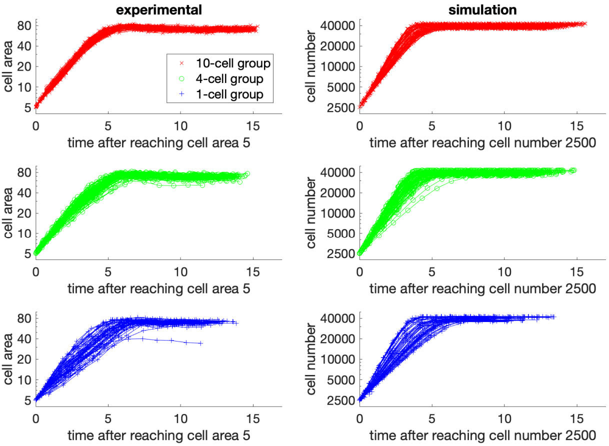


Figure: Translated population curves, starting from cell area 5 or cell number 2500. Y-axis is in log scale.

Section I.1: Model

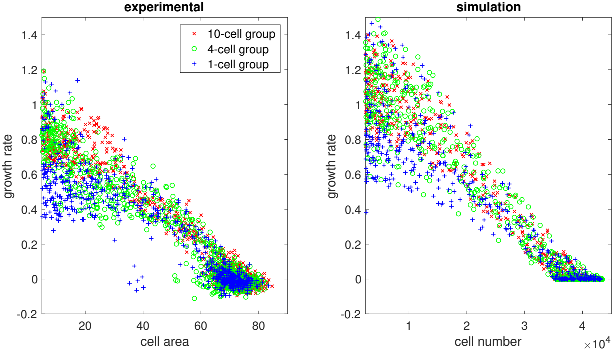


Figure: Growth rate versus population size.

Section I.1: Summary

- Experimental data reveal the existence of multiple states in cancer cell population.
- Corresponding model can reproduce experimental phenomena.

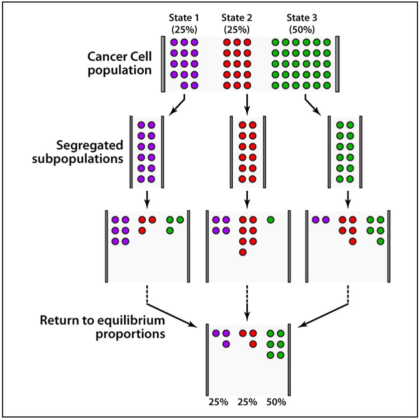
- Questions?

Section I.2: Multiple states

- The existence of multiple states has been verified in some other cancers.
- SUM159 breast cancer cell population has three states: stem, luminal, basal (distinguished by cell-surface markers).
- Why could multiple states (possibly with different growth rates) survive simultaneously?
- There exist epigenetic transitions between different states. Such change of state is inheritable.

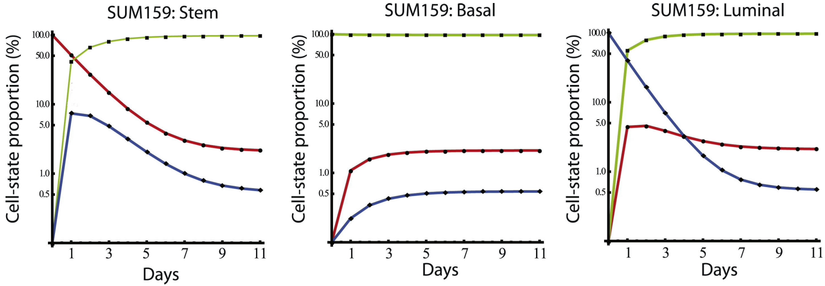
Section I.2: Multiple states

- Starting from any one state, other states will emerge, and the population gradually recovers the equilibrium proportions.
- It is called the “state equilibrium phenomenon”.



Section I.2: Experiments

How to explain such state equilibrium phenomenon?



Section I.2: Deterministic model

- Cells can divide, die or transform into other states. Assume cells do not interact, and there is no carrying capacity.
- The population vector $\vec{x}$ of different states satisfies a linear ODE system:

  $$\mathrm{d}\vec{x}/\mathrm{d}t = \vec{x}\mathbf{A},$$

  where $\mathbf{A} = \{a_{ij}\}$, the matrix of transition rates.
- The population proportion vector $\vec{w} = \vec{x}/||\vec{x}||_1$ satisfies a quadratic system:

  $$\frac{\mathrm{d}\vec{w}}{\mathrm{d}t} = \vec{w}[\mathbf{A} - (\vec{w}\vec{b}')\mathbf{I}],$$

  where $\vec{b} = \vec{1}\mathbf{A}'$.

Section I.2: Deterministic model

Perron-Frobenius Theorem states that **A** has a real eigenvalue λ_1, which is larger than the real parts of any other eigenvalues. Its normalized eigenvector is denoted by $\vec{u}_1$.

Theorem

If λ_1 is a simple root of the characteristic polynomial (in reality, this holds in general), the system $\mathrm{d}\vec{w}/\mathrm{d}t = \vec{w}[\boldsymbol{A} - (\vec{w}\vec{b}')\boldsymbol{I}]$ has a unique stationary fixed point $\vec{u}_1$.

Therefore the proportion vector $\vec{w}$ always converges to $\vec{u}_1$.

Section I.2: Stochastic model

- We can describe this population with a branching process.
- One cell of state i, Y_i, can branch into a (stochastic) combination of cells with different states: $Y_i \overset{\alpha_i}{\to} d_{i1} Y_1 + d_{i2} Y_2 + \cdots + d_{in} Y_n$. The waiting time is exponential with rate α_i.
- Here d_{ij} are random variables. For example,
  $d_{11} = 2$, $d_{12} = 0$ means division $Y_1 \to 2Y_1$;
  $d_{11} = d_{12} = 0$ means death $Y_1 \to \emptyset$;
  $d_{11} = 0$, $d_{12} = 1$ means transition $Y_1 \to Y_2$.
- If we take expectations for population, the branching process model returns to the ODE model.

Section I.2: Stochastic model

- Due to stochasticity, it is possible that all cells die out, and the proportions cannot be defined.
- We focus on the stochastic trajectories that no state dies out forever (called “non-extinction”).
- If $\lambda_1 > 0$, as the initial cell number increases, the probability of non-extinction tends to 1.

Theorem

Assume that $\lambda_1 > 0$ and λ_1 is a simple root of the characteristic polynomial. Conditioned on non-extinction, the proportion vector $\vec{w}$ converges to $\vec{u}_1$ with probability 1.

- This is a strong law of large numbers for branching processes. It improves a result by Svante Janson in 2004.
- It provides a stochastic explanation for the state equilibrium phenomenon.

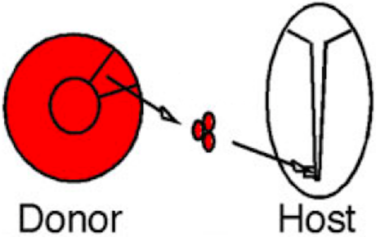
Section I.2: Summary

- We have explained the state equilibrium phenomenon in ODE model and branching process model.
- Questions?

Tissue Transplantation Experiments: Inference and Experimental Design

Section II: Outline

- Tissue transplantation experiments are important in developmental biology. However, most experimental results are unknown.
- Penalty function-based method to infer unknown experimental results.
- How to design experiments, so that the inference method can be applied most efficiently?



- Tissue transplantation experiments: For an embryo, excise a piece of one tissue (donor tissue), and transplant it to another tissue (host tissue).
- E.g., the transplantation experiment with donor tissue D and host tissue H is denoted as {D,H}.
- The transplanted tissue is placed in an unnatural environment. Therefore, its development might be normal (N) or abnormal (A).

Section II.1: Experiments

- Developmental biology: Why could a zygote (in natural environment) develop into an adult animal?
- To understand why the developmental process in natural environment works, we also need to understand why the developmental process in unnatural environment does not always work.
- Tissue transplantation experiments describe how tissues behave in unnatural environments.

Section II.1: Experiments

		Donor						
		AM19	PM19	PM15	UL11	LL11	LL15	LL19
Host	AM19	?	N	A	A	A	A	N
	PM19	?	N	?	N	N	?	?
	PM15	?	?	?	?	?	?	?
	UL11	?	N	?	N	N	?	?
	LL11	?	N	?	N	N	?	?
	LL15	?	?	?	?	?	?	?
	LL19	?	?	?	?	?	?	?

Table: Results for *Xenopus laevis*, reported by Krneta-Stankic et al. 2010

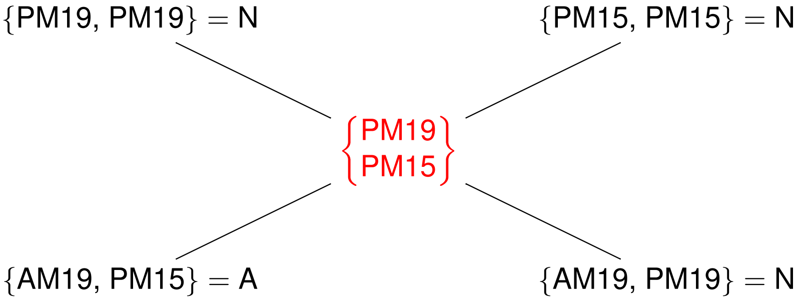
N=normal; A=abnormal; ?=unknown. AM=anterior paraxial mesoderm; PM=presomitic mesoderm; UL=upper lateral lip; LL=lower lip; Number=developmental stage.

Section II.1: Experiments

- There are many possible tissue transplantation experiments. Only a small portion has been conducted. We need a method to infer the unknown results.
- Core idea: Similar experiments should have similar results. For similar experiments, we can use known results to infer unknown results.
- Assume we have known the similarities between experiments.

Section II.1: Ideas

Experiment similarity chart:



$\{\text{PM19, PM19}\} = \text{N}$

$\{\text{PM15, PM15}\} = \text{N}$

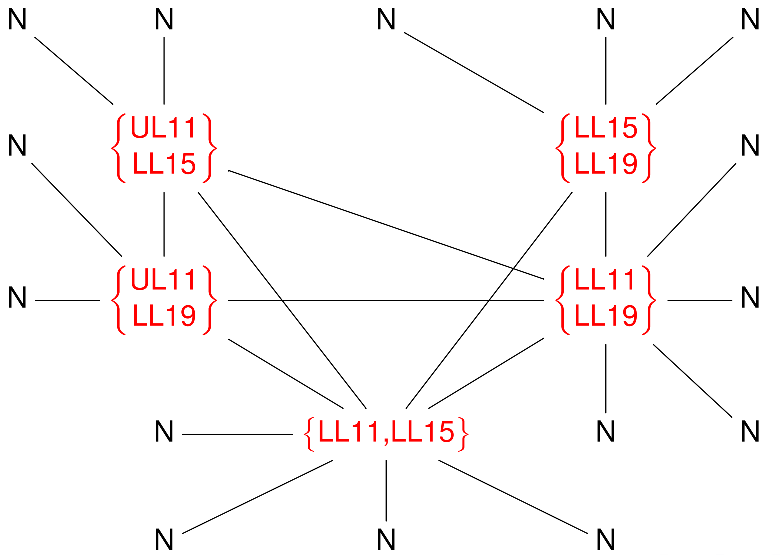
$\begin{Bmatrix}\text{PM19}\\ \text{PM15}\end{Bmatrix}$

$\{\text{AM19, PM15}\} = \text{A}$

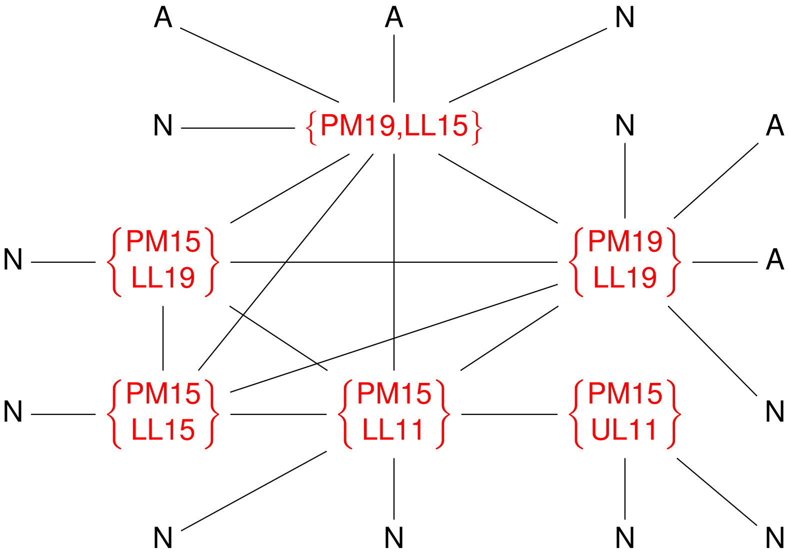
$\{\text{AM19, PM19}\} = \text{N}$

Black/red terms are experiments with known/unknown results.
Linked experiments are similar.
The result of {PM19,PM15} can be inferred by the known results of similar experiments.

Section II.1: Ideas



Section II.1: Ideas



Section II.1: Ideas

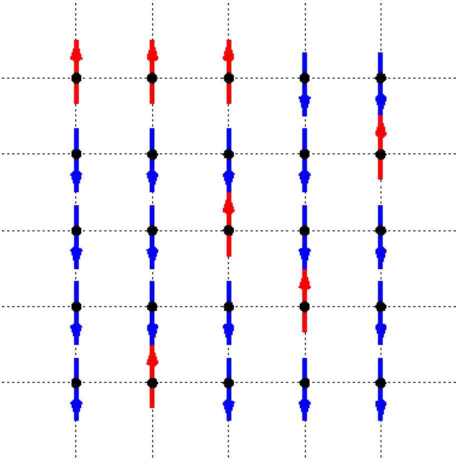
- How to determine the similarities between experiments?
- We can describe the similarities between tissues qualitatively or quantitatively. (Transcriptome information, concentration of certain molecules, distance on the developmental tree, etc.)
- With the similarities between tissues, we can establish the similarities between experiments. Experiments are similar if they have similar donor tissues and similar host tissues.
- For this project, we do not have enough data. Thus the experiment similarities are assigned subjectively and rather arbitrarily.

Section II.1: Ideas

- We take guesses of unknown experimental results, and use a penalty function to evaluate such guesses. Then we can find the best guesses.
- There is a penalty if two similar experiments have different results.
- For the concrete form of this penalty function, we can get inspirations from the Ising model.

Section II.1: Ising model

The Ising model describes ferromagnetism in statistical mechanics. Consider a set of lattice sites, where each site k has a variable σ_k that takes $+1$ or -1.



Section II.1: Ising model

- For a configuration σ of ± 1, its energy function (no external field) is
$$\mathrm{H}(\sigma) = -\sum_{i \sim j} J_{ij} \sigma_i \sigma_j,$$
where $i \sim j$ means site i and site j are neighboring, and $J_{ij} \geq 0$ is the interaction coefficient. For neighboring sites i, j, when $\sigma_i = \sigma_j$, the energy is lower.
- The probability of a configuration σ is
$$\mathbb{P}_\beta(\sigma) = e^{-\beta \mathrm{H}(\sigma)} / Z_\beta,$$
where $\beta = (k_B T)^{-1}$, Z_β is the normalization constant.
- Configuration with lower energy (smaller penalty) has higher probability. Neighboring sites tend to have the same value.

Section II.1: Ideas

Analogies between tissue transplantation experiments and the Ising model:

Tissue transplantation	Ising model
Experiment similarity chart	Lattice
Experiment	Site
Similar experiments	Neighboring sites
Result: normal/abnormal	Value: +1/-1
Penalty: similar experiments have different results	Penalty: neighboring sites have different values
Penalty function?	Energy function

Pure analogies, not physical correspondence.

Section II.1: Ideas

- For tissue transplantation experiments, we take guesses for unknown experimental results $\{\sigma_i\}$.
- The penalty function is

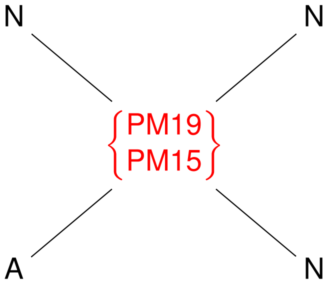
$$\mathrm{H}(\sigma) = -\sum_{i,j} J_{ij}\sigma_i\sigma_j.$$

- The probability of a configuration σ is

$$\mathbb{P}_\beta(\sigma) = e^{-\beta\mathrm{H}(\sigma)}/Z_\beta.$$

- Regard N as $+1$, and A as -1.
- Use experiment similarities to determine parameter J_{ij}.

Section II.1: Ideas



N N

$\left\{\begin{matrix}\text{PM19}\\\text{PM15}\end{matrix}\right\}$

A N

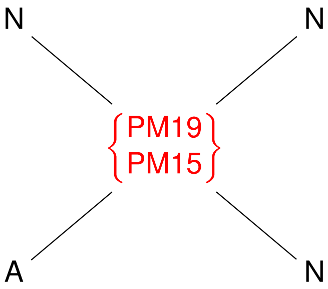
$$\mathrm{H}(\sigma) = -\sum_{i,j} J_{ij}\sigma_i\sigma_j,\ \mathbb{P}_\beta(\sigma) = e^{-\beta\mathrm{H}(\sigma)}/Z_\beta.$$

$\{\text{PM19}, \text{PM15}\} = \text{N}$:

$$\mathrm{H} = -1\times 1\times 1 - 1\times 1\times 1 - 1\times 1\times(-1) - 1\times 1\times 1 = -2.$$

$$\mathbb{P} = e^{-0.1\times(-2)}/Z_\beta = 0.60.$$

Section II.1: Ideas



$$\mathrm{H}(\sigma) = -\sum_{i,j} J_{ij}\sigma_i\sigma_j,\ \mathbb{P}_\beta(\sigma) = e^{-\beta \mathrm{H}(\sigma)}/Z_\beta.$$

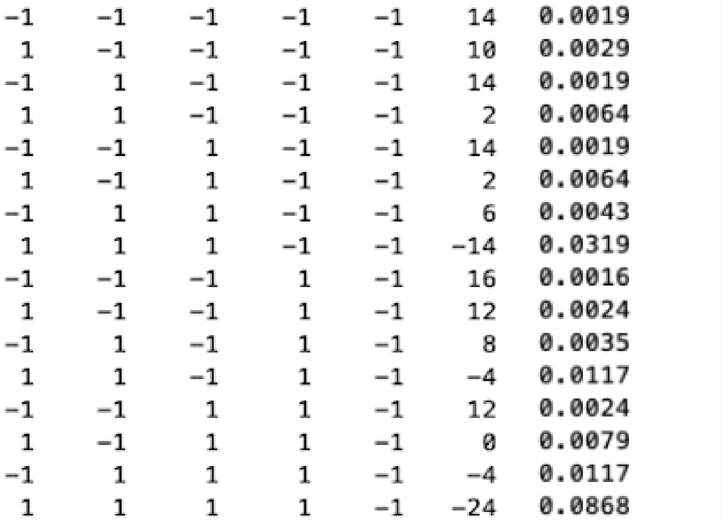
$\{\text{PM19}, \text{PM15}\} = \text{A}$:

$$\mathrm{H} = -1\times(-1)\times1-1\times(-1)\times1-1\times(-1)\times(-1)-1\times(-1)\times1 = 2.$$

$$\mathbb{P} = e^{-0.1\times2}/Z_\beta = 0.40.$$

Result=N is the most probable guess. $\mathbb{P}(\text{N}) = 0.60$.

Section II.1: Ideas



Configuration of guesses					Penalty	Probability
-1	-1	-1	-1	-1	14	0.0019
1	-1	-1	-1	-1	10	0.0029
-1	1	-1	-1	-1	14	0.0019
1	1	-1	-1	-1	2	0.0064
-1	-1	1	-1	-1	14	0.0019
1	-1	1	-1	-1	2	0.0064
-1	1	1	-1	-1	6	0.0043
1	1	1	-1	-1	-14	0.0319
-1	-1	-1	1	-1	16	0.0016
1	-1	-1	1	-1	12	0.0024
-1	1	-1	1	-1	8	0.0035
1	1	-1	1	-1	-4	0.0117
-1	-1	1	1	-1	12	0.0024
1	-1	1	1	-1	0	0.0079
-1	1	1	1	-1	-4	0.0117
1	1	1	1	-1	-24	0.0868

Section II.1: Ideas

For each configuration of the unknown results (guesses), we can calculate its probability. We can determine the most probable guesses:

		Donor						
		AM19	PM19	PM15	UL11	LL11	LL15	LL19
Host	AM19	*N*	N	A	A	A	A	N
	PM19	*N*	N	N	N	N	N	N
	PM15	*A*	N	*N*	N	N	N	N
	UL11	*A*	N	N	N	N	N	N
	LL11	*A*	N	N	N	N	N	N
	LL15	*A*	N	N	N	N	*N*	N
	LL19	*N*	N	N	N	N	N	*N*

Section II.1: Ideas

Since each configuration of guesses has a probability, we can take expectations, and obtain the probability for each experimental result to be “N”:

					Donor			
		AM19	PM19	PM15	UL11	LL11	LL15	LL19
Host	AM19	*100%*	100%	0%	0%	0%	0%	100%
	PM19	*100%*	100%	65%	100%	100%	49%	56%
	PM15	*0%*	65%	*100%*	62%	62%	53%	54%
	UL11	*0%*	100%	62%	100%	100%	81%	81%
	LL11	*0%*	100%	62%	100%	100%	90%	90%
	LL15	*0%*	49%	53%	81%	90%	*100%*	86%
	LL19	*100%*	56%	54%	81%	90%	86%	*100%*

Section II.1: Another situation

- For now, we have designed an inference method that works for experiments with binary deterministic results.
- What if the known experimental results are not deterministic, but stochastic?

Section II.1: Another situation

		Donor				
		PLE11	PLE12	PLE14	PLE16	PLE19
	LFR\PLE14	61%	58%	82%	?	?
Host	LFR\PLE16	?	?	?	?	?
	LFR\PLE19	4%	24%	83%	?	100%

Table: Results for *Xenopus laevis*, reported by Henry et al. 1987

- Percentage is the probability of normal development (N).
- PLE11: presumptive lens ectoderm, stage 11.
- LFR\PLE14: lens-forming region without presumptive lens ectoderm, stage 14.

Section II.1: Another situation

- Sample deterministic configurations from these stochastic results (assume different experiments are independent).
- For each deterministic configuration, apply our method to obtain the expectation of guesses.
- For example, assume we have three similar experiments: [61%N ? 58%N].

Sample deterministic results: $\mathbb{P}([\text{N ? N}]) = 61\% \times 58\% = 35\%$.

Apply the inference method: $\mathbb{P}(\text{?=N} \mid [\text{N ? N}]) = 98\%$.

$$\mathbb{P}([\text{N N N}]) = \mathbb{P}([\text{N ? N}]) \times \mathbb{P}(\text{?=N} \mid [\text{N ? N}]) = 35\% \times 98\% = 35\%.$$

Section II.1: Another situation

Similarly, we can calculate for other deterministic configurations of [61%N ? 58%N]:

$$\mathbb{P}([\text{N ? A}]) = 61\% \times (100\% - 58\%) = 26\%.$$

$$\mathbb{P}([\text{N N A}]) = 26\% \times 50\% = 13\%.$$

$$\mathbb{P}([\text{A ? N}]) = (100\% - 61\%) \times 58\% = 23\%.$$

$$\mathbb{P}([\text{A N N}]) = 23\% \times 50\% = 11\%.$$

$$\mathbb{P}([\text{A ? A}]) = (100\% - 61\%) \times (100\% - 58\%) = 16\%.$$

$$\mathbb{P}([\text{A N A}]) = 16\% \times 2\% = 0\%.$$

Then average over these deterministic configurations:

$$\mathbb{P}([\text{?=N}]) = \mathbb{P}([\text{N N N}])+\mathbb{P}([\text{N N A}])+\mathbb{P}([\text{A N N}])+\mathbb{P}([\text{A N A}]) = 59\%.$$

Final results: [61%N 59%N 58%N].

Section II.1: Another situation

		Donor				
		PLE11	PLE12	PLE14	PLE16	PLE19
	LFR\PLE14	61%	58%	82%	?	?
Host	LFR\PLE16	?	?	?	?	?
	LFR\PLE19	4%	24%	83%	?	100%

Table: Results for *Xenopus laevis*, reported by Henry et al. 1987

		Donor				
		PLE11	PLE12	PLE14	PLE16	PLE19
	LFR\PLE14	61%	58%	82%	93%	94%
Host	LFR\PLE16	39%	53%	88%	97%	97%
	LFR\PLE19	4%	24%	83%	96%	100%

Table: Inferred results

Section II.1: Yet another situation

- The methods are for experiments with binary results.
- What if the known experimental results are not binary?
- The penalty function is:

$$\mathrm{H}(\sigma) = -\sum_{i\sim j} J_{ij}\sigma_i\sigma_j.$$

  The cross term $\sigma_i\sigma_j$ measures the similarity between σ_i and σ_j.
- Rewrite the penalty function:

$$\mathrm{H}(\sigma) = -\sum_{i\sim j} J_{ij} f(\sigma_i, \sigma_j).$$

- If σ_i, σ_j are more similar, $f(\sigma_i, \sigma_j)$ is larger. Also, $f(\sigma_i, \sigma_j) = f(\sigma_j, \sigma_i)$. For binary case, $f(\sigma_i, \sigma_j) = \sigma_i\sigma_j$.

Section II.1: Summary

- Based on the similarities between experiments, we have designed methods to infer the unknown experimental results.
- The results are not necessarily deterministic or binary.
- In the future, we hope to have more experimental data to verify the inference results and determine the parameters.
- Such methods should not be limited to tissue transplantation experiments.

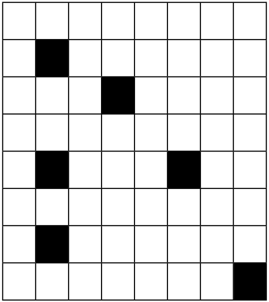
- Questions?

Section II.2: Experimental design

- Assume there are many tissue transplantation experiments, and we do not have any results yet.
- To know all the results, we can choose some experiments to conduct, and apply our method to infer the unknown experimental results.
- How to choose experiments to conduct?

Section II.2: Experimental design

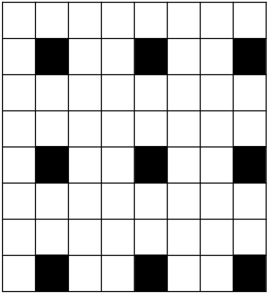
- Assume the experiment similarity chart is 2-D lattice. Each unit is an experiment, and neighboring units are similar experiments.
- Black units are conducted experiments, and white units are non-conducted experiments.
- Experimental design (choosing experiments to conduct) becomes coloring the chart.

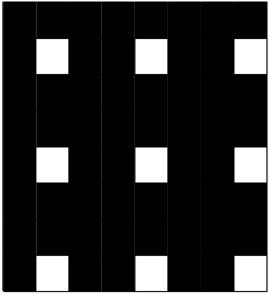


	■						
			■				
	■				■		
	■						
							■

Section II.2: Experimental design

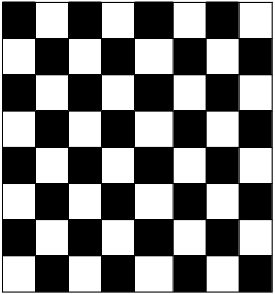
We need enough data to apply the inference method. We should minimize the experimental cost.



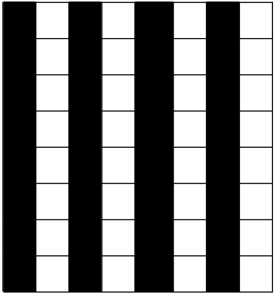


Section II.2: Experimental design

- The results of non-conducted experiments are inferred by similar conducted experiments.
- To guarantee the inference quality, one non-conducted experiment should be similar to at least k conducted experiments.



$k = 4$



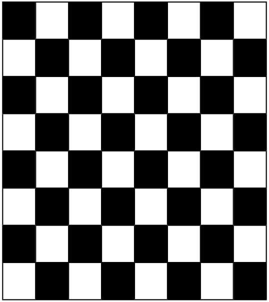
$k = 2$

Section II.2: Experimental design

- If one non-conducted experiment is similar to at least k conducted experiments, how to minimize the number of conducted experiments?
- The most efficient design: no conducted experiments are similar, and each non-conducted experiment is similar to exactly k conducted experiments.
- How to color the 2-D square lattice $\mathbb{Z}^2$, so that two black units are not neighboring, and each white unit is neighboring to exactly k black units?

Section II.2: Experimental design

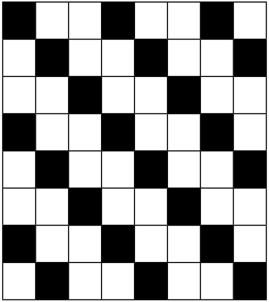
No neighboring black units, and each white unit is neighboring to $k = 4$ black units (ignore the boundary cases).



We need to conduct $1/2$ experiments.

Section II.2: Experimental design

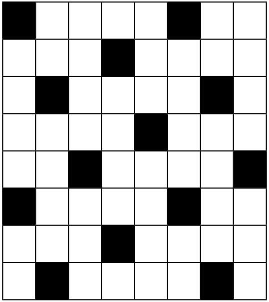
No neighboring black units, and each white unit is neighboring to $k = 2$ black units (ignore the boundary cases).



We need to conduct $1/3$ experiments.

Section II.2: Experimental design

No neighboring black units, and each white unit is neighboring to $k = 1$ black units (ignore the boundary cases).



We need to conduct $1/5$ experiments.

Section II.2: Experimental design

- In practice, the experiment similarity chart is not 2-dimensional, but 4-dimensional. Each experiment has a coordinate (x, y, z, w) that stands for donor tissue type, donor tissue developmental stage, host tissue type, host tissue developmental stage.
- For now, we assume the chart is $\mathbb{Z}^4$.
- Similar coloring problems for such 4-dimensional figures.
- We need some abstract methods.

Section II.2: Experimental design

- In $\mathbb{Z}^4$, each unit is neighboring to 8 units.
- For $k = 8$, color a unit (x, y, z, w) if

$$x + y + z + w \equiv 0 \mod 2.$$

  We need to conduct $1/2$ experiments.

- For $k = 4$, color a unit (x, y, z, w) if

$$x + y + z + w \equiv 0 \mod 3.$$

  We need to conduct $1/3$ experiments.

- For $k = 2$, color a unit (x, y, z, w) if

$$x + 2y + z + 2w \equiv 0 \mod 5.$$

  We need to conduct $1/5$ experiments.

Section II.2: Experimental design

- For $k = 1$, color a unit (x, y, z, w) if

$$x + 2y + 3z + 4w \equiv 0 \mod 9.$$

  We need to conduct $1/9$ experiments.
- Such methods can be generalized to $\mathbb{Z}^n$ and k that $k \mid 2n$.
- Color a unit $(x_1, \dots, x_n)$ if $a_1 x_1 + \cdots + a_n x_n \equiv 0 \mod (2n/k + 1)$. Here if $k \mid n$, $(a_1, \dots, a_n)$ are k groups of $1, 2, \dots, n/k$; otherwise, $(a_1, \dots, a_n)$ are $k/2$ groups of $1, 2, \dots, 2n/k$.

Section II.2: Experimental design

- In practice, $k = 2$ or $k = 1$ is enough to conduct satisfactory inference. Therefore we only need to conduct $1/5 - 1/3$ experiments (two-dimensional) or $1/9 - 1/5$ experiments (four-dimensional).
- We have solved a problem: how to choose experiments to conduct, so that other unknown results can be inferred properly with minimal cost.
- In mathematical biology, sometimes the right key (mathematics) is unexpected.

- Questions?

Conclusion

- Reveal the existence of multiple states in cancer cell population, and prove the state equilibrium phenomenon with ODEs and branching processes.
- Develop methods to infer the results of tissue transplantation experiments, and solve the experimental design problem with combinatorics.
- The same flavor: analyze biological data; build models; extract and solve meaningful mathematical problems.

Thank you!

Other related works

Related works

- Mathematical biology.
- Design algorithms to find “jumping genes” in gene sequences. Design algorithms to calculate the distance between developmental trees.
- Analyze the notion of “positional information” in developmental biology. Build models for embryo development.
- Ongoing: Infer the structure of gene regulatory networks. Build models for cell membrane electric potential.

Related works

- Applied probability (especially in machine learning theory).
- Causal inference: Impossibilities in quantifying causal relationships.
- Reinforcement learning: Policy evaluation in pricing processes with historical data (possibly polluted).
- Statistical physics: Entropy production of lifted Markov chains.