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The simulation of tumor heterogeneity and cancer-cell evolution using hallmarks approach

The program code and the results of testing

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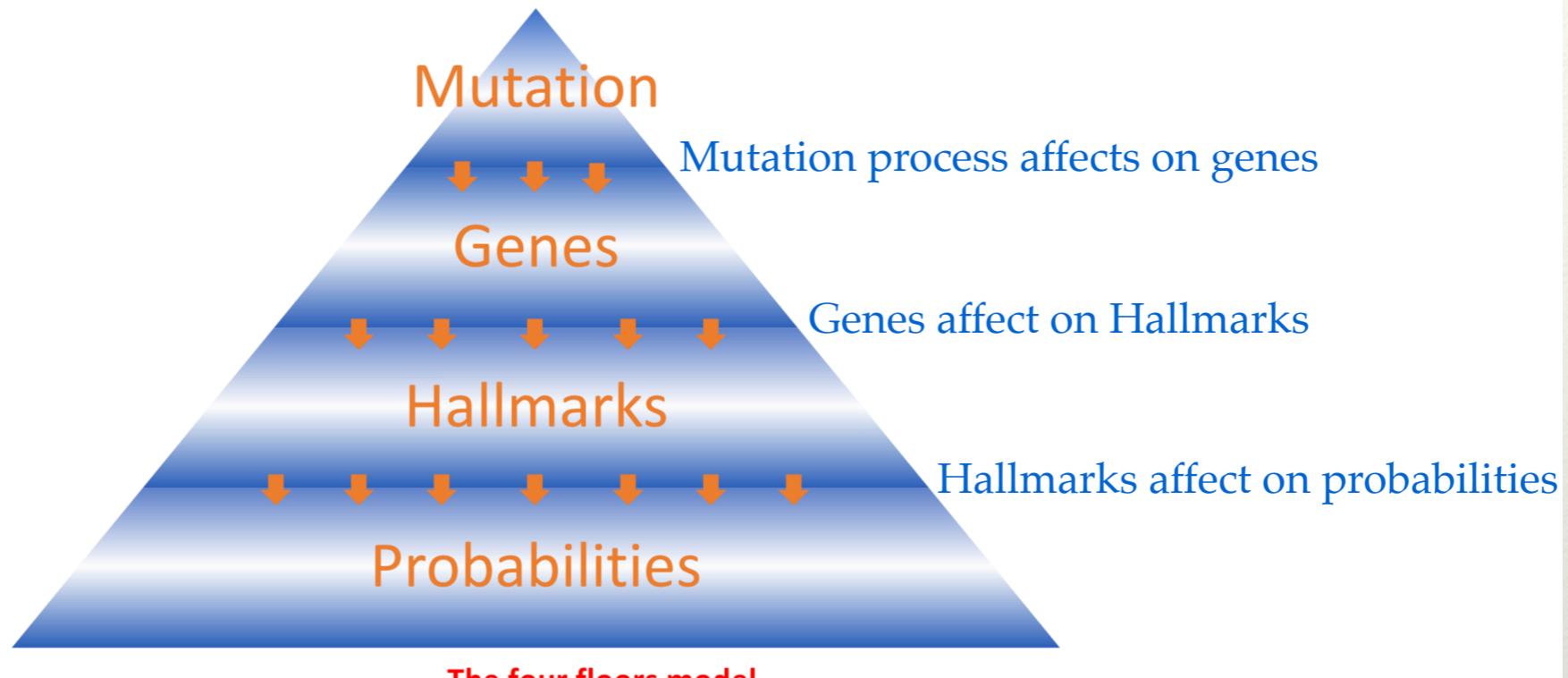
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Goal and tasks of simulation

- ❖ **GOAL:** to research and simulate the heterogeneous group of cancer cells with gene effect
- ❖ *Tasks:*
- ❖ to design the tests for program code
- ❖ to design the service for output data and instruments for analyzation of results

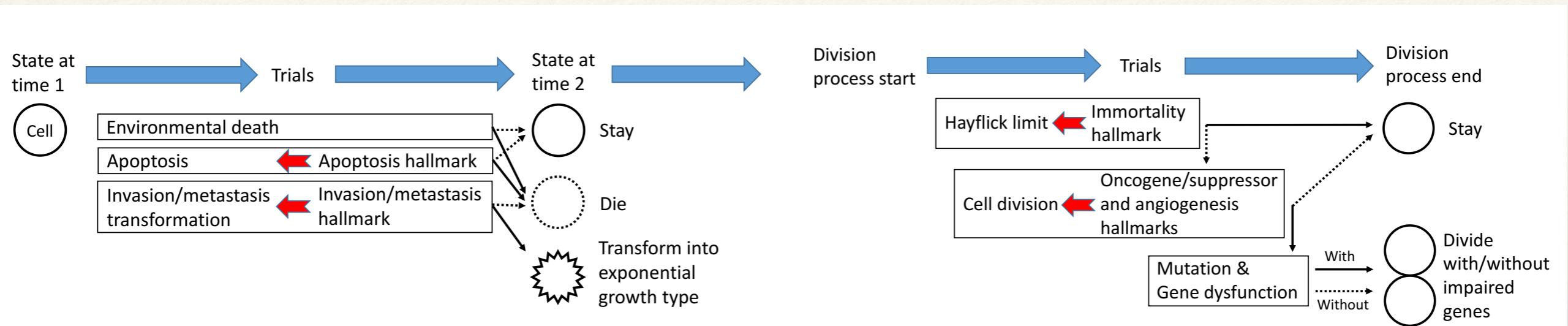
“The four floors model”



MUTATION						
<i>Ga</i>	<i>Destroyed Genes / ALL Genes</i>	<i>Gi</i>	<i>Gim</i>	-	<i>Gd</i>	<i>Gb</i>
$H_a = \sum_k (G_a)_k$	<i>x-mutation density</i>	$H_i = \sum_k (G_i)_k$	$H_{im} = \sum_k (G_{im})_k$	-	$H_d = \sum_k (G_d)_k$	$H_b = \sum_k (G_b)_k$
<i>Apoptosis</i> $a' = a - H_a$ $a = \frac{1}{1 + e^{-s_0(x-0.5)}}$	<i>Hayflick limit (immortalization)</i> $i' = 1 - H_i$	<i>Invasion/metastasis transformation</i> $im' = H_{im}$	<i>Environmental death</i> k'	<i>Division process</i> $d' = \begin{cases} d - E' \cdot N & , \text{ for normal cells} \\ d & , \text{ for metastasis cells} \end{cases}$ $E' = \frac{E_0}{1 + F_0 \cdot H_b}$ and $d = H_d$ N – number of normal cells		

Model for Cancer Cell Evolution

proposed by prof. Mamoru Kato



The oncogene/suppressor hallmark variable, H_d , for example:

$$H_d = w_1^d \cdot g_1^d + w_2^d \cdot g_2^d + w_3^d \cdot g_3^d + w_4^d \cdot g_4^d$$

$$\text{Let } (w_1^d, w_2^d, w_3^d, w_4^d) = (0.1, 0.2, 0.3, 0.4)$$

$$\text{When } (g_1^d, g_2^d, g_3^d, g_4^d) = (1, 1, 0, 0)$$

$$H_d = 0.1 \cdot 1 + 0.2 \cdot 1 + 0.3 \cdot 0 + 0.4 \cdot 0 = 0.3$$

H_d interferes with a probability value of the cell division trial:

$$d' = d_0 + H_d = 0 + 0.3 = 0.3$$

The simplest model to examine the effects of hallmarks themselves

$$\begin{bmatrix} H_a \\ H_{im} \\ H_i \\ H_d \\ H_b \end{bmatrix} = \begin{bmatrix} w_1^a \cdot g_1^a \\ w_1^{im} \cdot g_1^{im} \\ w_1^i \cdot g_1^i \\ w_1^d \cdot g_1^d \\ w_1^b \cdot g_1^b \end{bmatrix} = \begin{bmatrix} 1 \cdot I_a \\ 1 \cdot I_{im} \\ 1 \cdot I_i \\ 1 \cdot I_d \\ 1 \cdot I_b \end{bmatrix}$$

Implementation of model: input parameters

Variable type	Notation	Description	Per	Interfered by hallmarks	Time change	Notation as parameter	Possible initial values for parameter	
Cell	c	Cell division counter	-	No	Dynamic	-	-	
	C_{max}	Maximum cell division number by Hayflic limit	-	No	Static	$(c_{max}, 0)$	50	
	k	Probability of cell death by environments		No	Static	k_0	{0.1, 0.2, ..., 0.9}	
	d	Cell division rate		Yes	Dynamic	(d_0)	0.1 for , arbitrarily time unit	
	i_m	Probability of invasion/metastasis transformation		Yes	Dynamic	-	-	
	a	Probability of cell death by apoptosis		Yes	Dynamic	s_0	{10, 15, 20, 30, 40, 90}	
	i	Probability of cell division stop by Hayflic limit		Yes	Dynamic	(i_0)	1	
	m	Mutation rate per bp	division	No	Static	m_0	{ 10^{-6} , 10^{-7} , $\underline{10^{-8}}$, 10^{-9} , 10^{-10} }	
	u_o	Probability of dysfunction of a oncogene	mutation	No	Static	$u_{o,0}$	{1/1, 1/10, 1/100}	
	u_s	Probability of dysfunction of a suppressor	mutation	No	Static	$u_{s,0}$	{1/1, 1/10, 1/100}	
External	N/M	Number of cells with logistic/exponential growth	-	No	Dynamic	-	-	
	E	Environmental resource limitation	-	No	Static	E_0	{ 10^{-1} , 10^{-2} , $\underline{10^{-3}}$, 10^{-4} , 10^{-5} }	
	F	Reduction effect to E by angiogenesis	-	No	Static	F_0	{ $\underline{10^1}$, 10^2 , $\underline{10^3}$, $\underline{10^6}$, $\underline{10^9}$ }	
	T	Time counter	-	-	-	-	-	

The “condition” column represents the condition when the trial is applied. Variables with prime (‘) in the “probability” column are effective (actually used) probabilities. Hs represent hallmark variables. σ is a sigmoid function.

Impaired_gene_density represents the fraction of impaired genes.

Gene file and Hallmark's weights

Gene file format

Column	Contents
1	Gene name
2	CDS length
3	Distinction of oncogene/ suppressor o: oncogene s: suppressor ?: unknown (will be randomly assigned)
4	Hallmark name: apoptosis immortalization growth anti-growth angiogenesis invasion
5	Hallmark weights. * For convenience, hallmark weights are automatically generated in random (rbeta) when this column is blank.

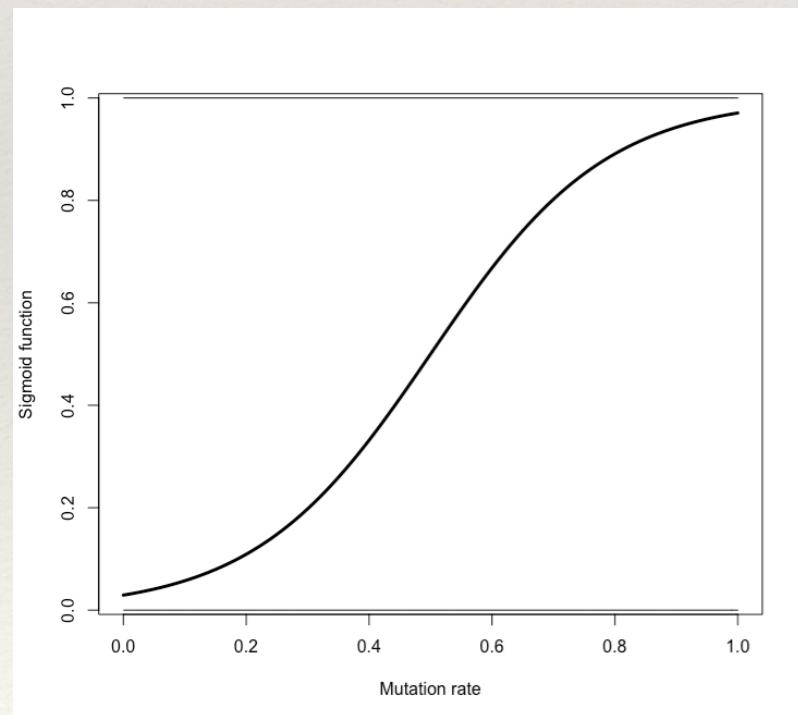
Example of gene file with Hallmark's weights

Gene name	CDS length	Distinct	Hallmark name	Hallmark weights
GA	1	o	apoptosis	1
GB	10	o	angiogenesis	1
GIM	20	s	invasion	1
GI	30	o	immortalization	1
GD1	40	?	growth	0.9
GD2	1	o	anti-growth	0.1

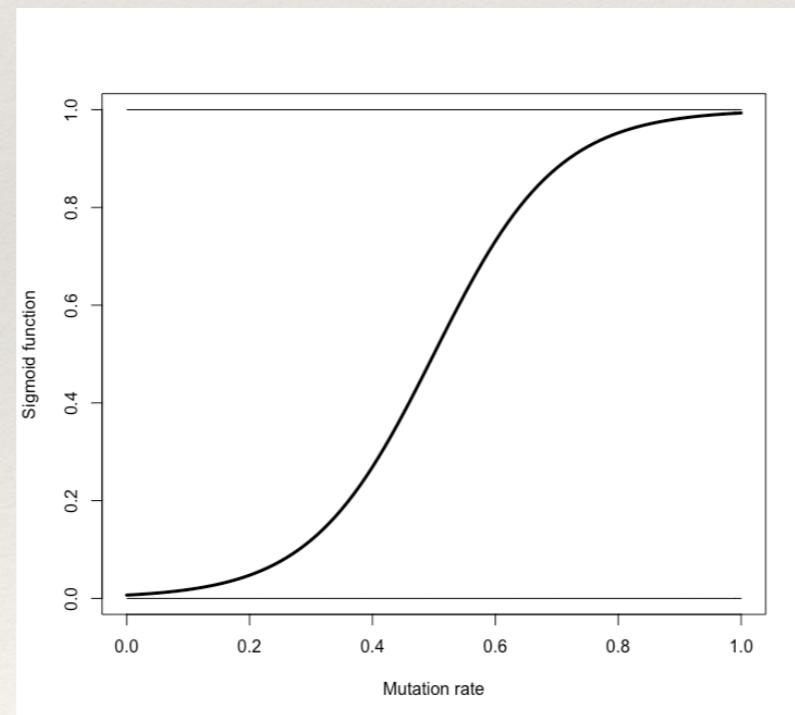
The sigmoid function

$$s(x) = \frac{1}{1 + e^{-s_0 \cdot (x - 0.5)}}$$

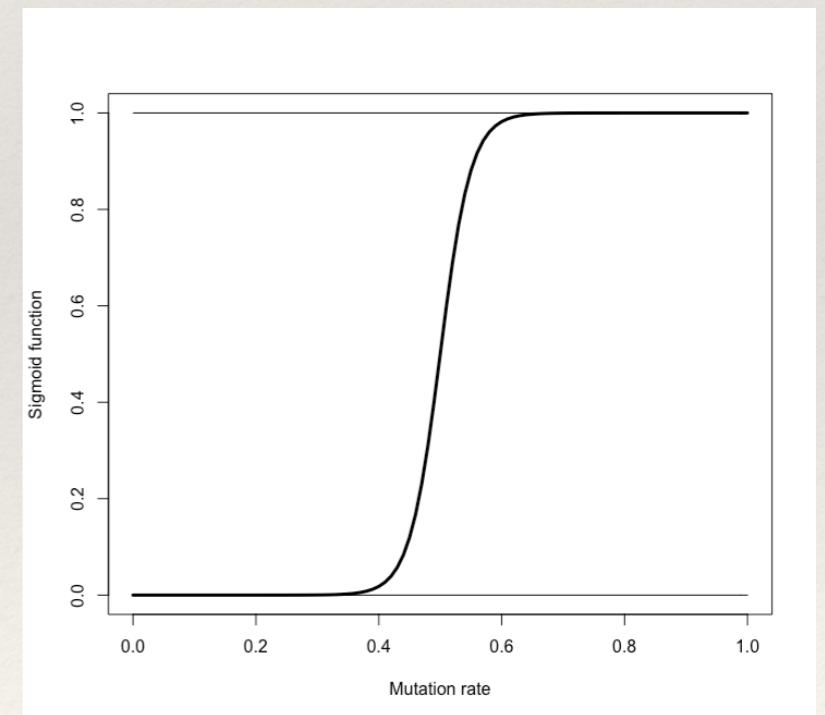
$$s_0 = 7$$



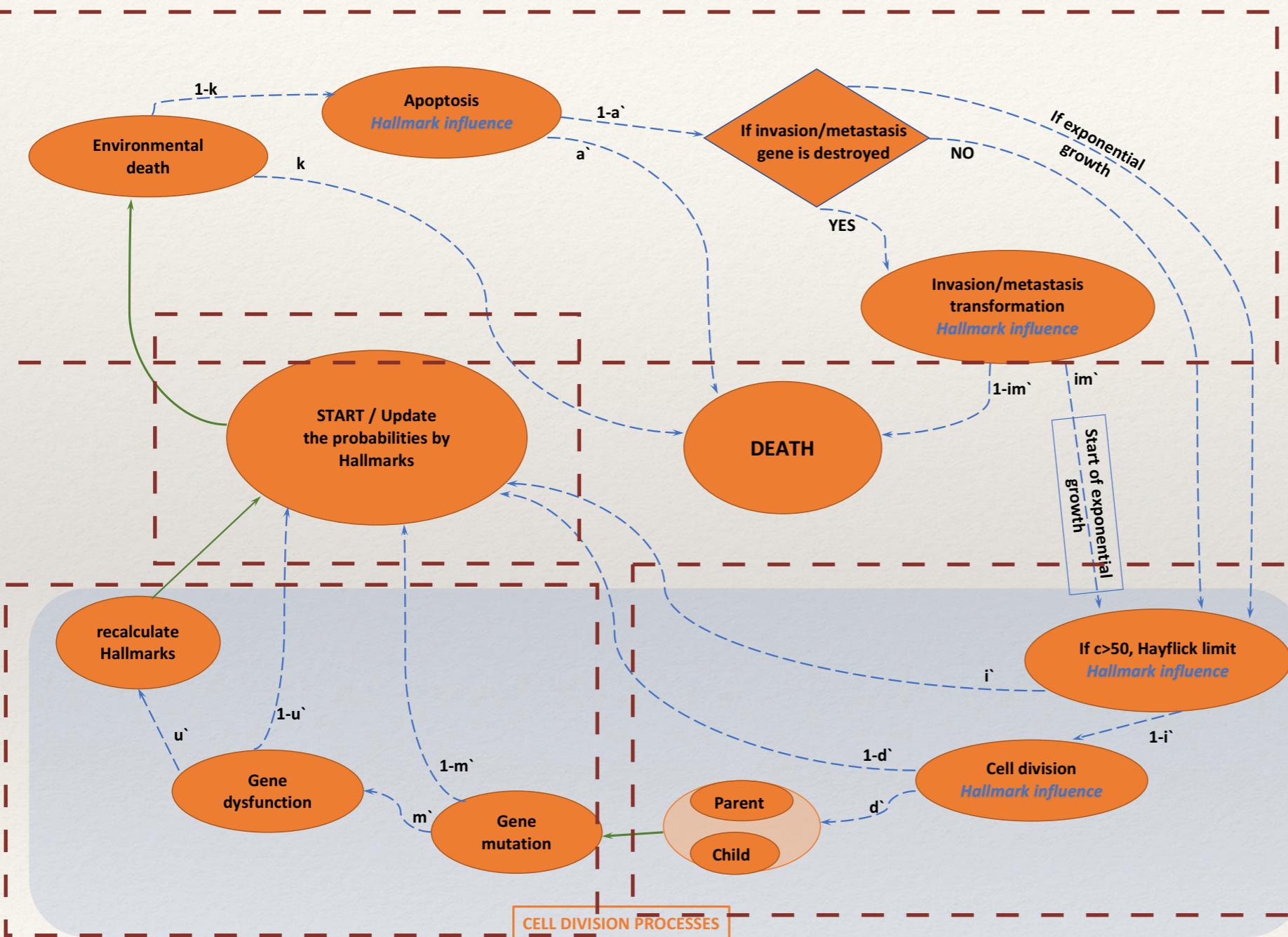
$$s_0 = 10$$



$$s_0 = 40$$

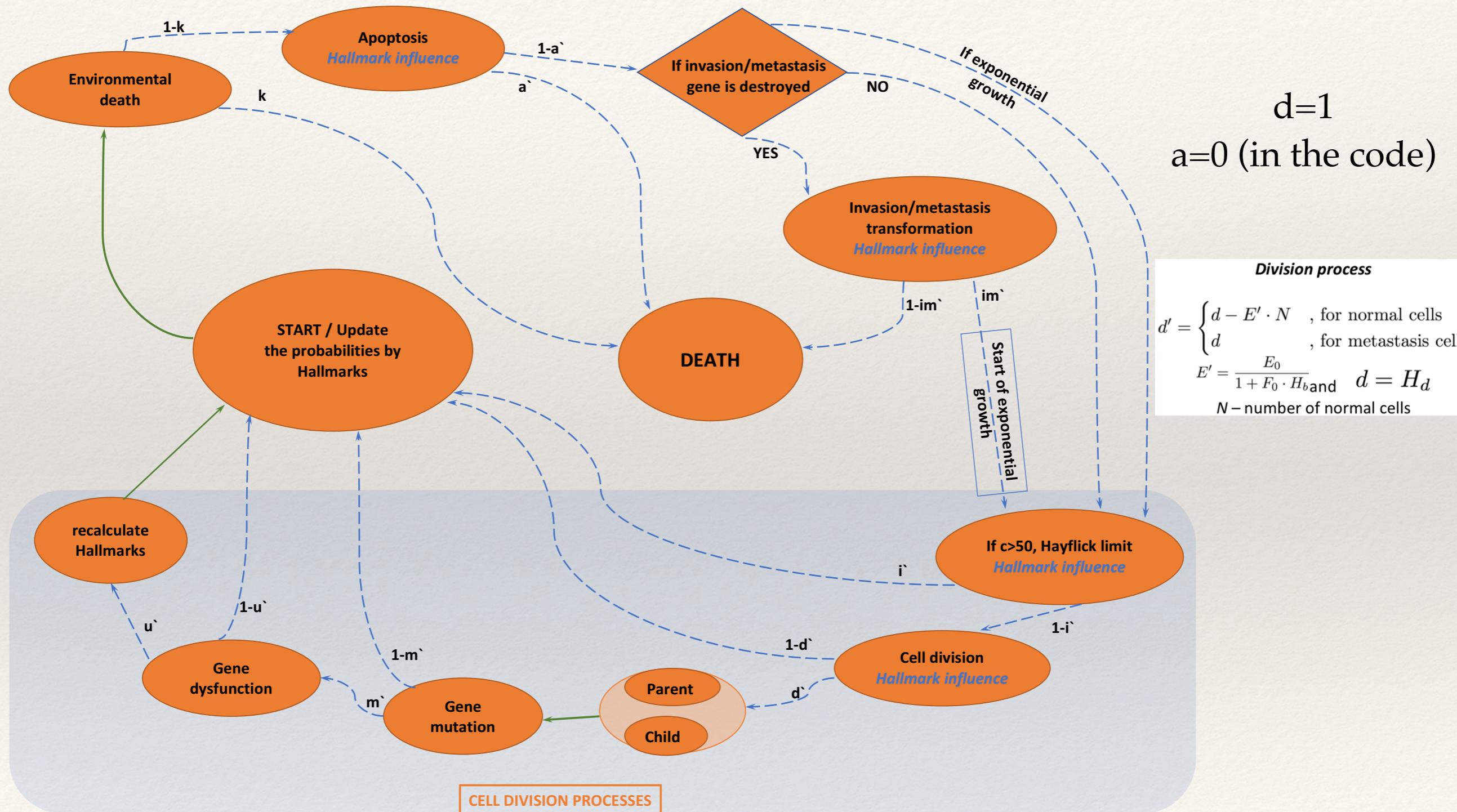


Scheme of simulation with processes and states

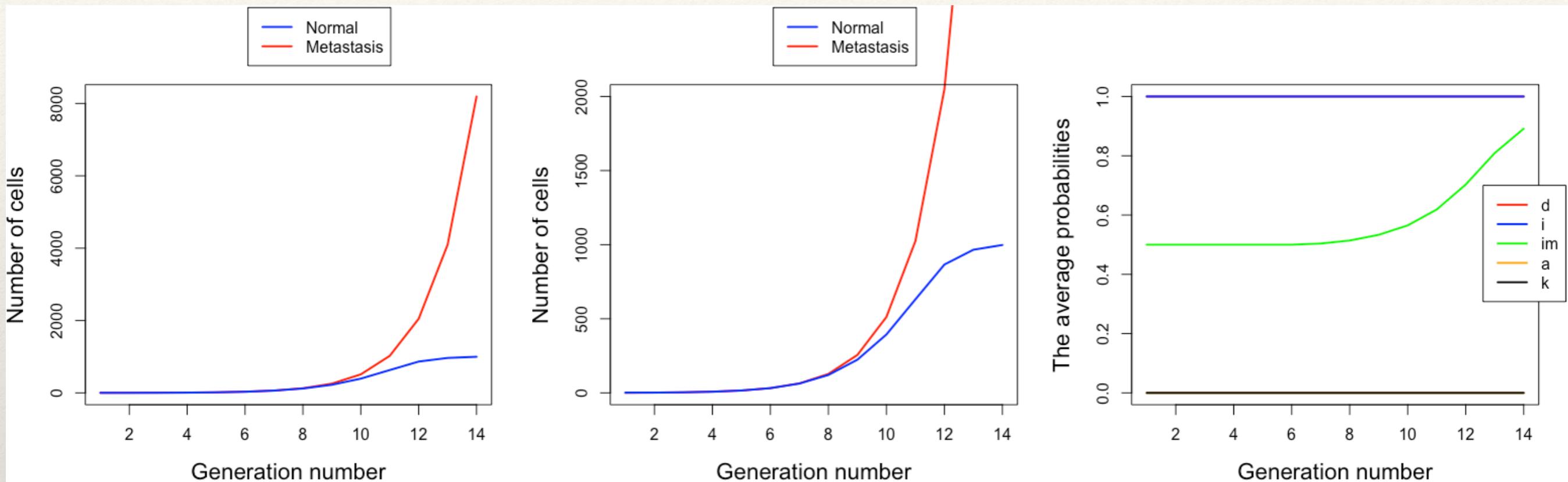


- Three types of tests:
1. For probabilities.
 2. For Hallmarks influence on probabilities.
 3. For the mutation process.

Test 1: division process without Hallmarks influence



Test 1: division process: results of simulation



Division process

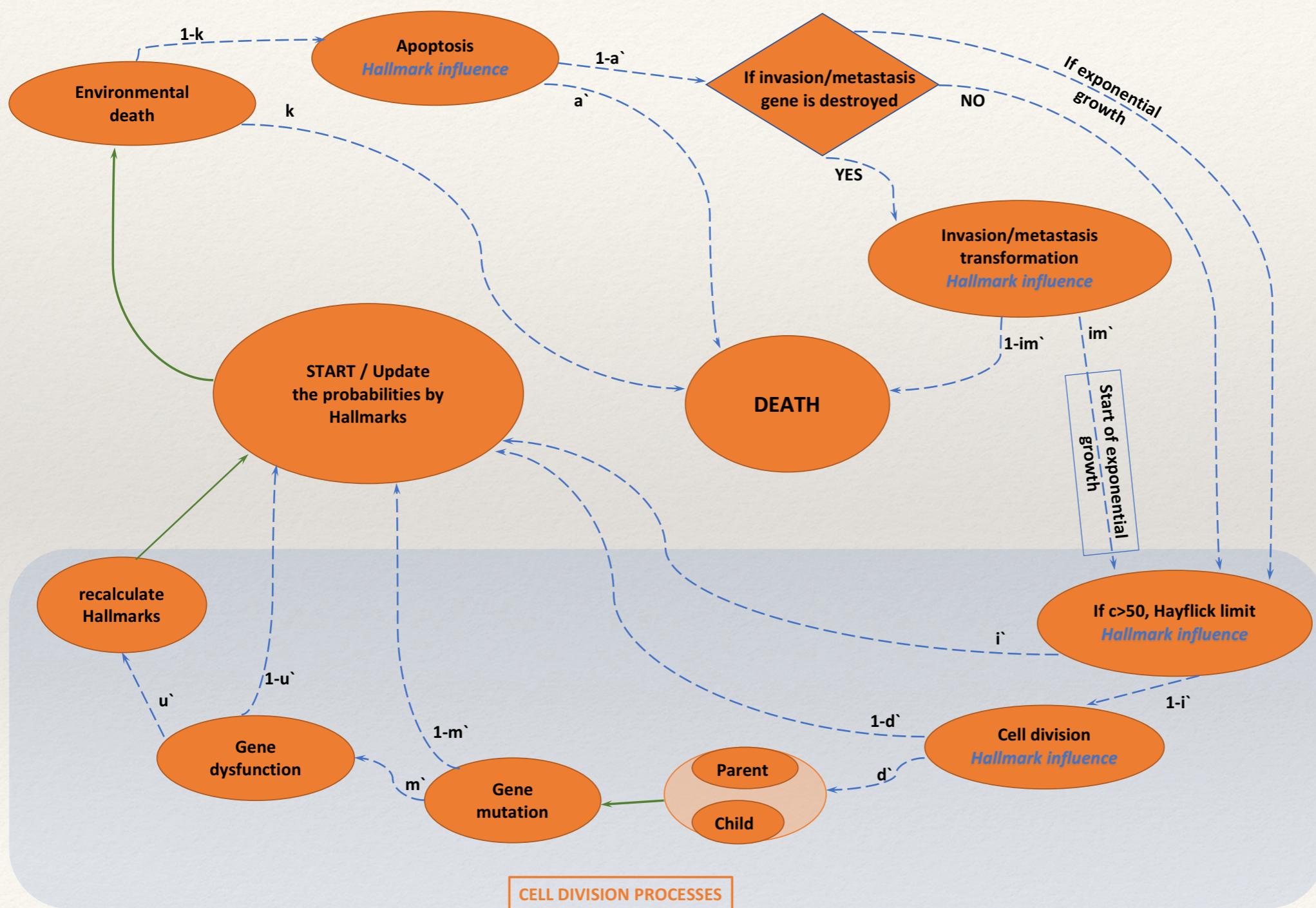
$$d' = \begin{cases} d - E' \cdot N & , \text{for normal cells} \\ d & , \text{for metastasis cells} \end{cases}$$

$$E' = \frac{E_0}{1 + F_0 \cdot H_b} \text{ and } d = H_d$$

N – number of normal cells

d=1
 a=0 (in the code)
 E=0.001
 F=1

Test 2: Environmental death

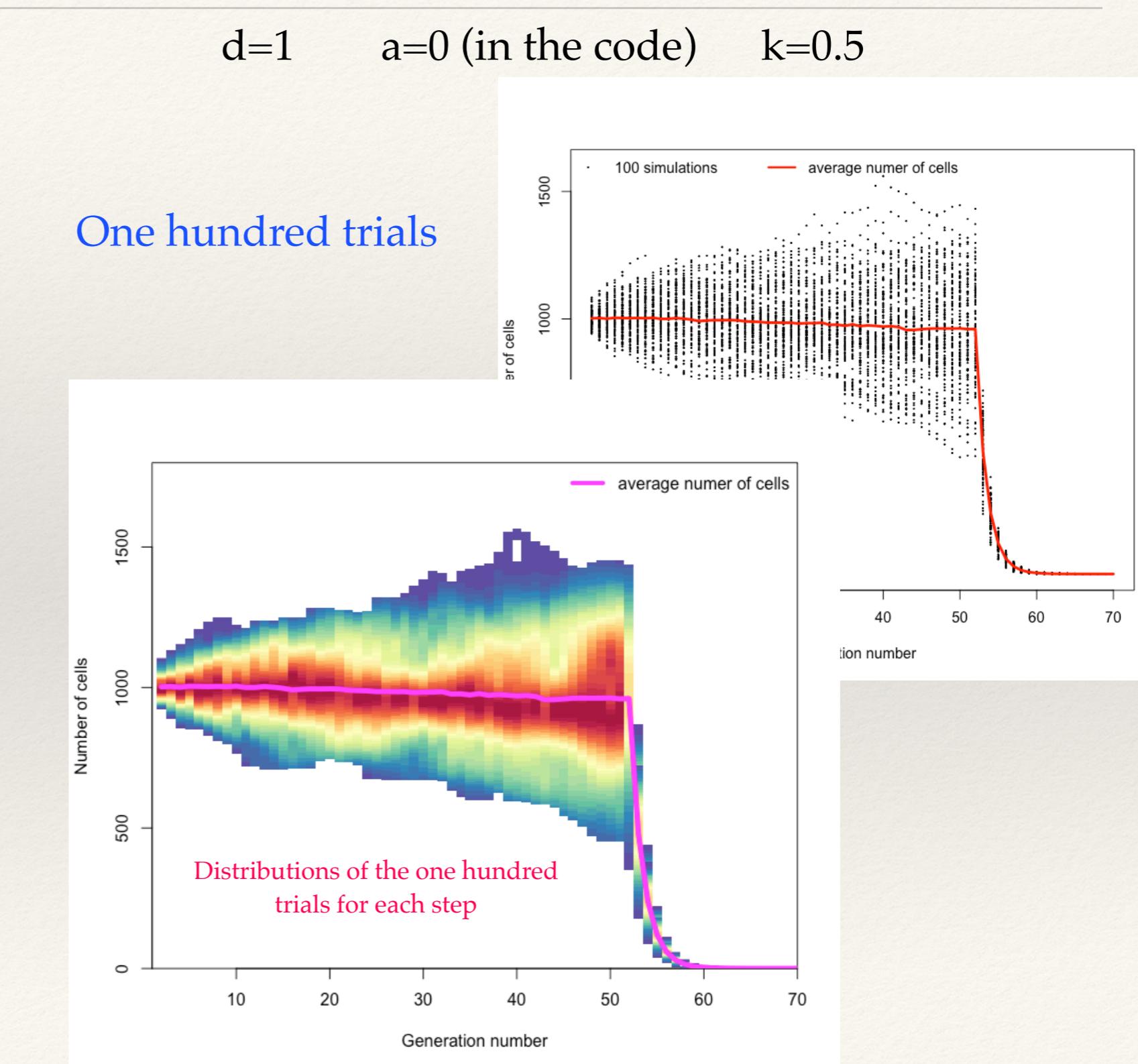
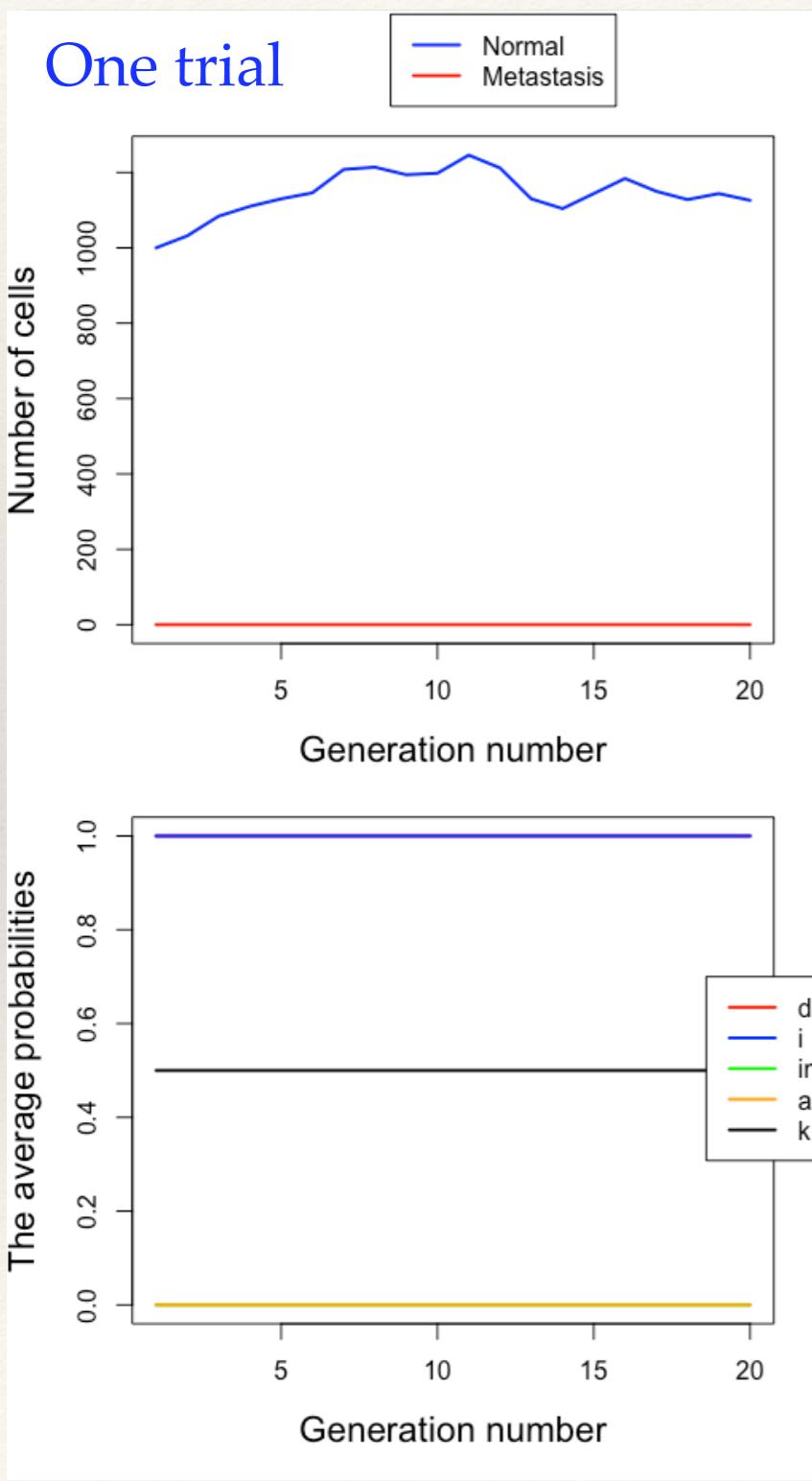


$$d=1$$

$$a=0 \text{ (in the code)}$$

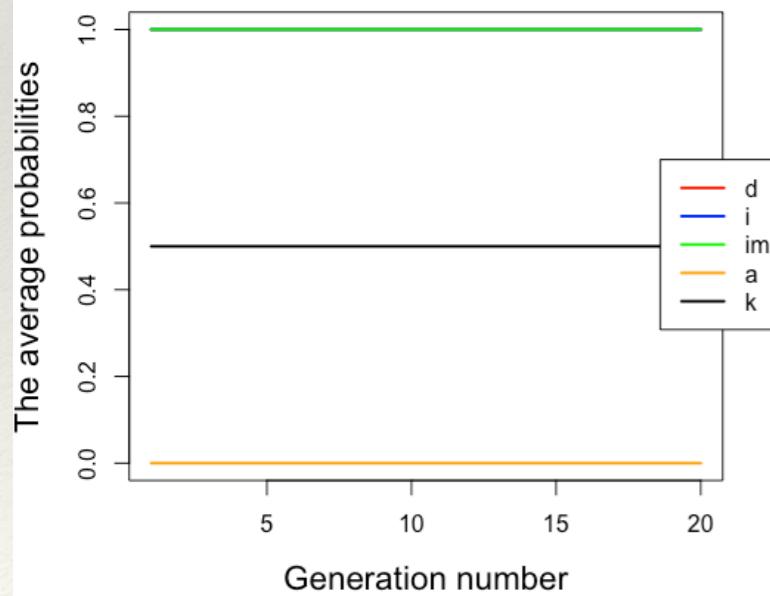
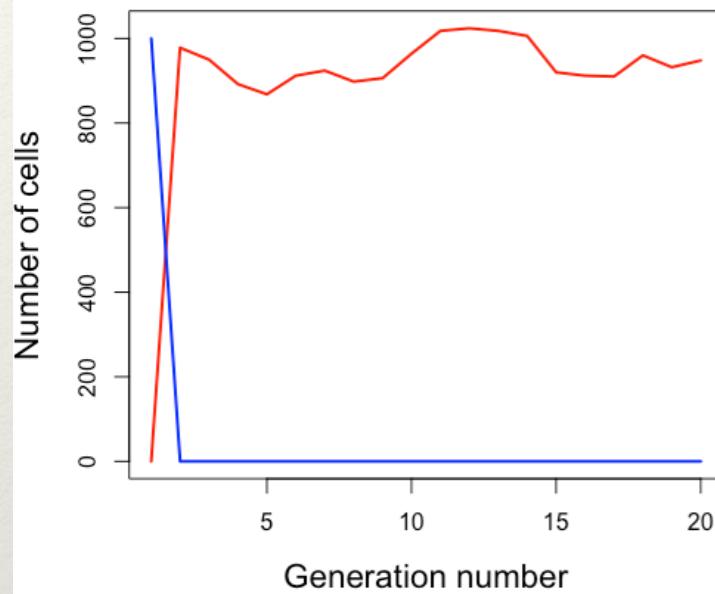
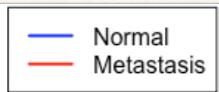
$$k=0.5$$

Test 2: Environmental death: results for normal cells



Test 2: Environmental death: results for metastasis cells

One trial

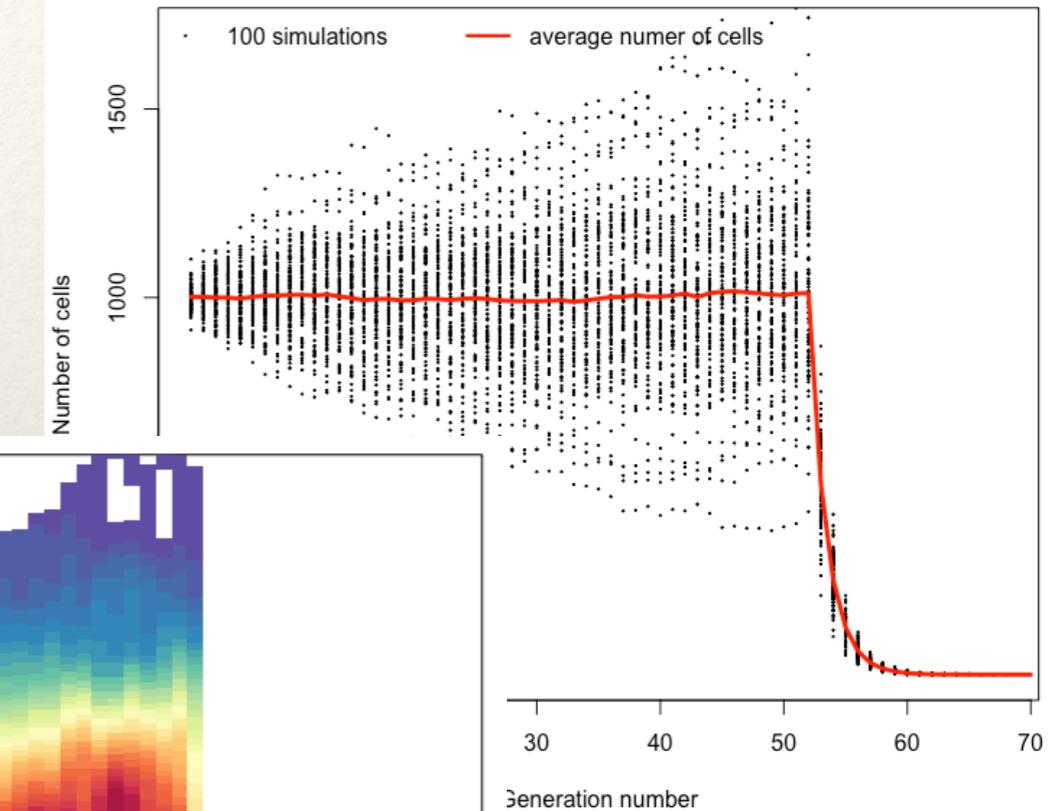
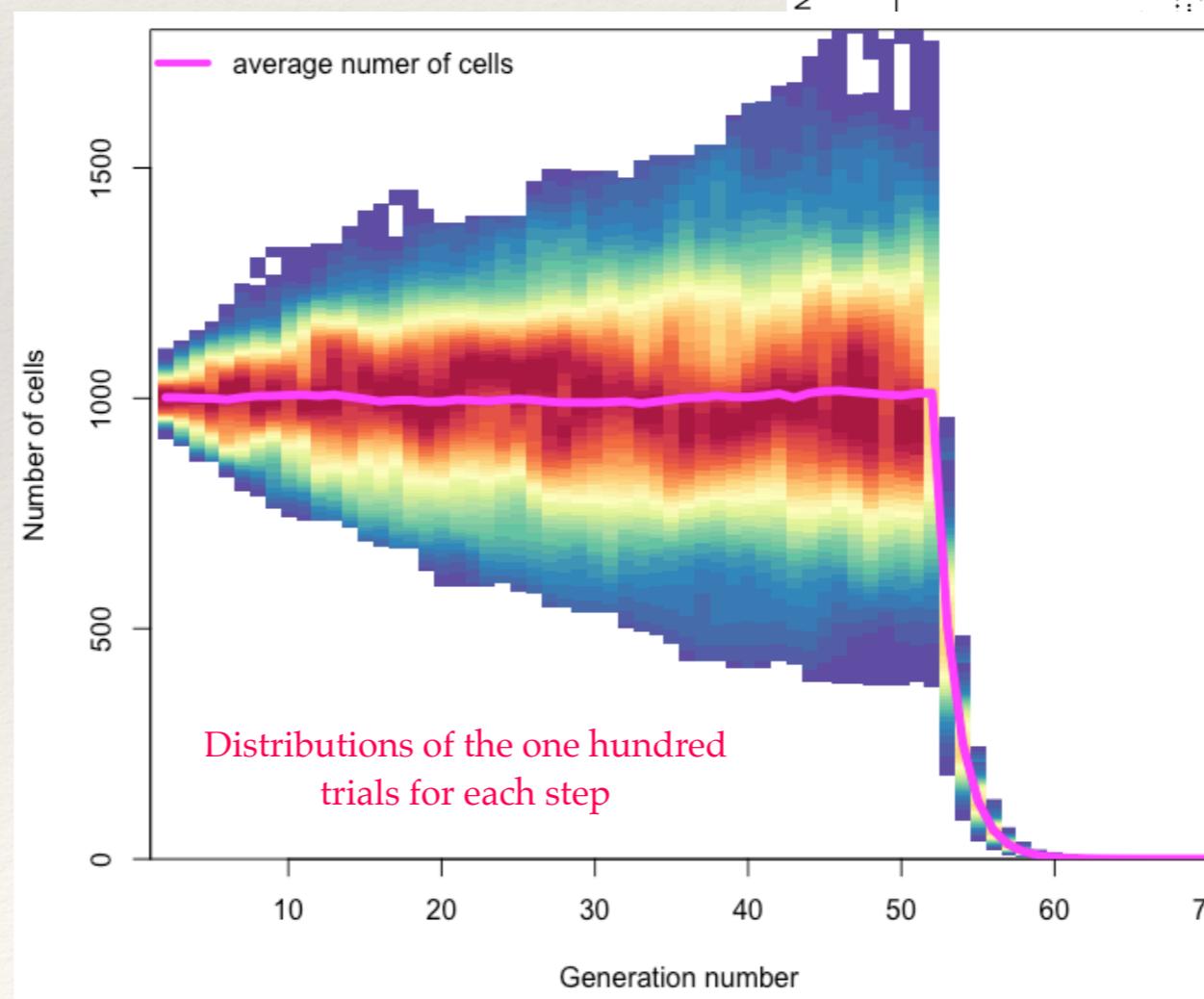


$d=1$

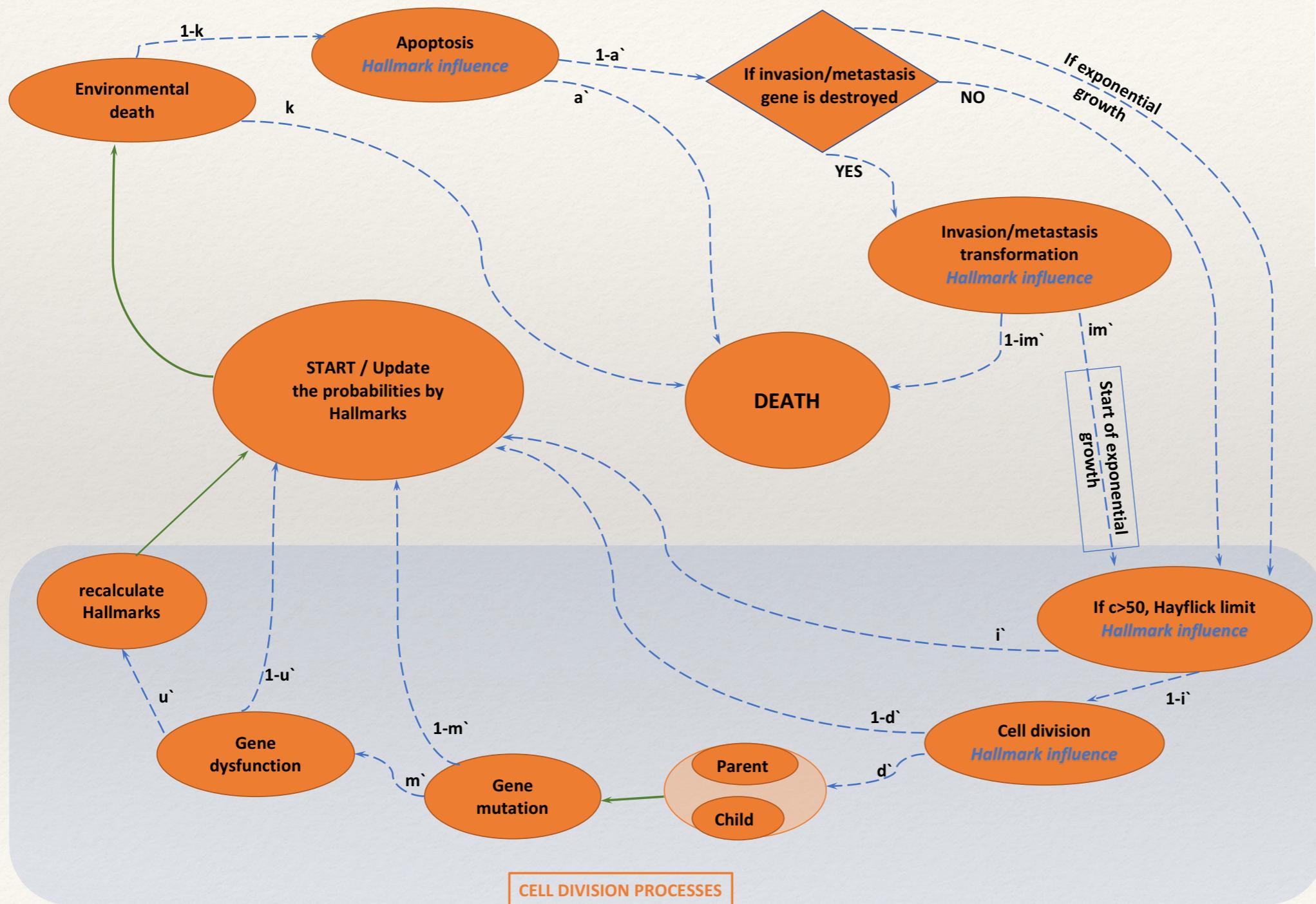
$a=0$ (in the code)

$k=0.5$

One hundred trials



Test 3: Apoptosis death: the results



Apoptosis

$$a' = a - H_a$$

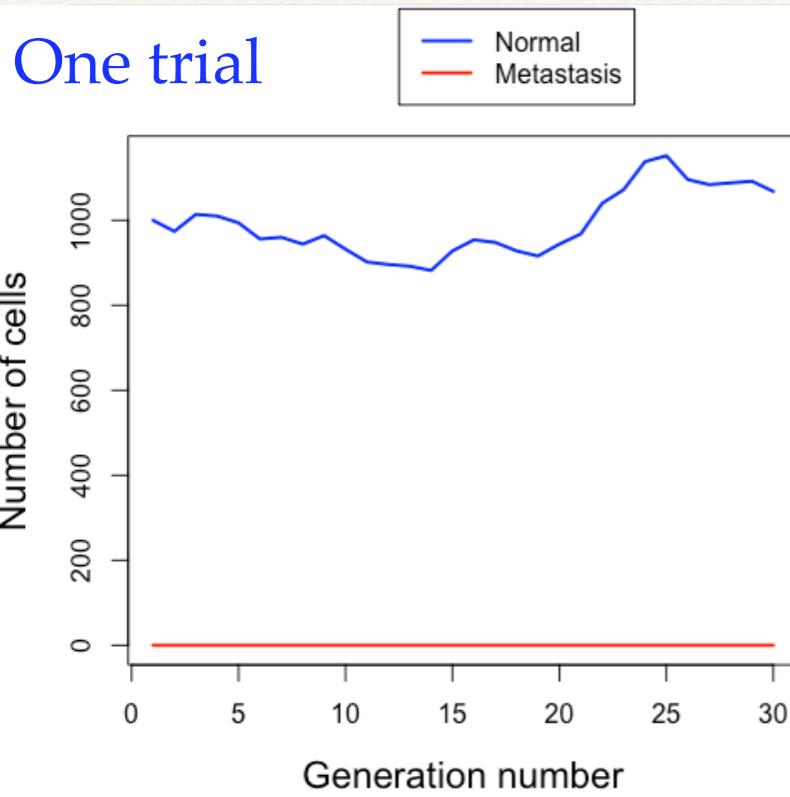
$$a = \frac{1}{1 + e^{-s_0(x-0.5)}}$$

$d=1$

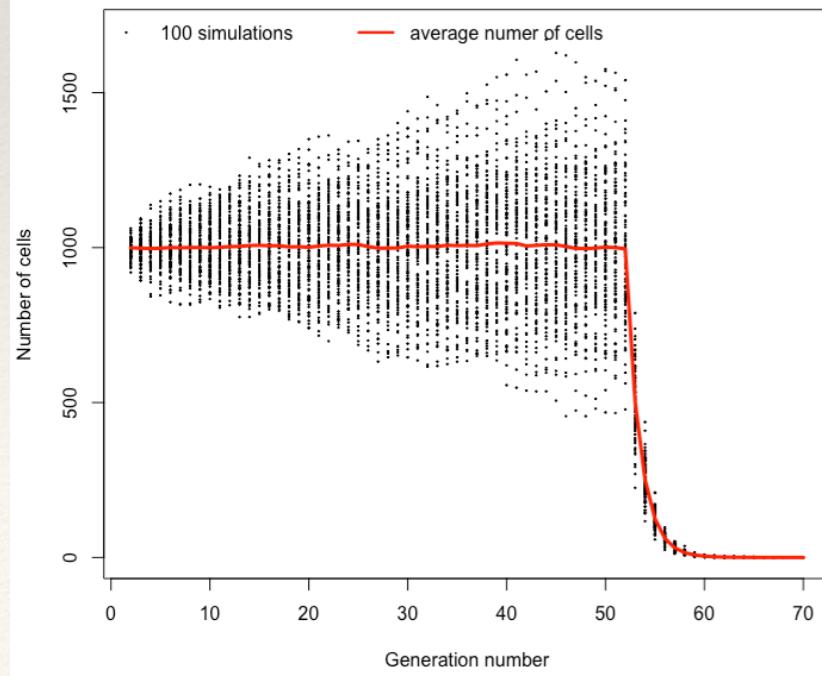
$a=0.5$ (in the code)

Test 3: Apoptosis death: Normal cells

One trial



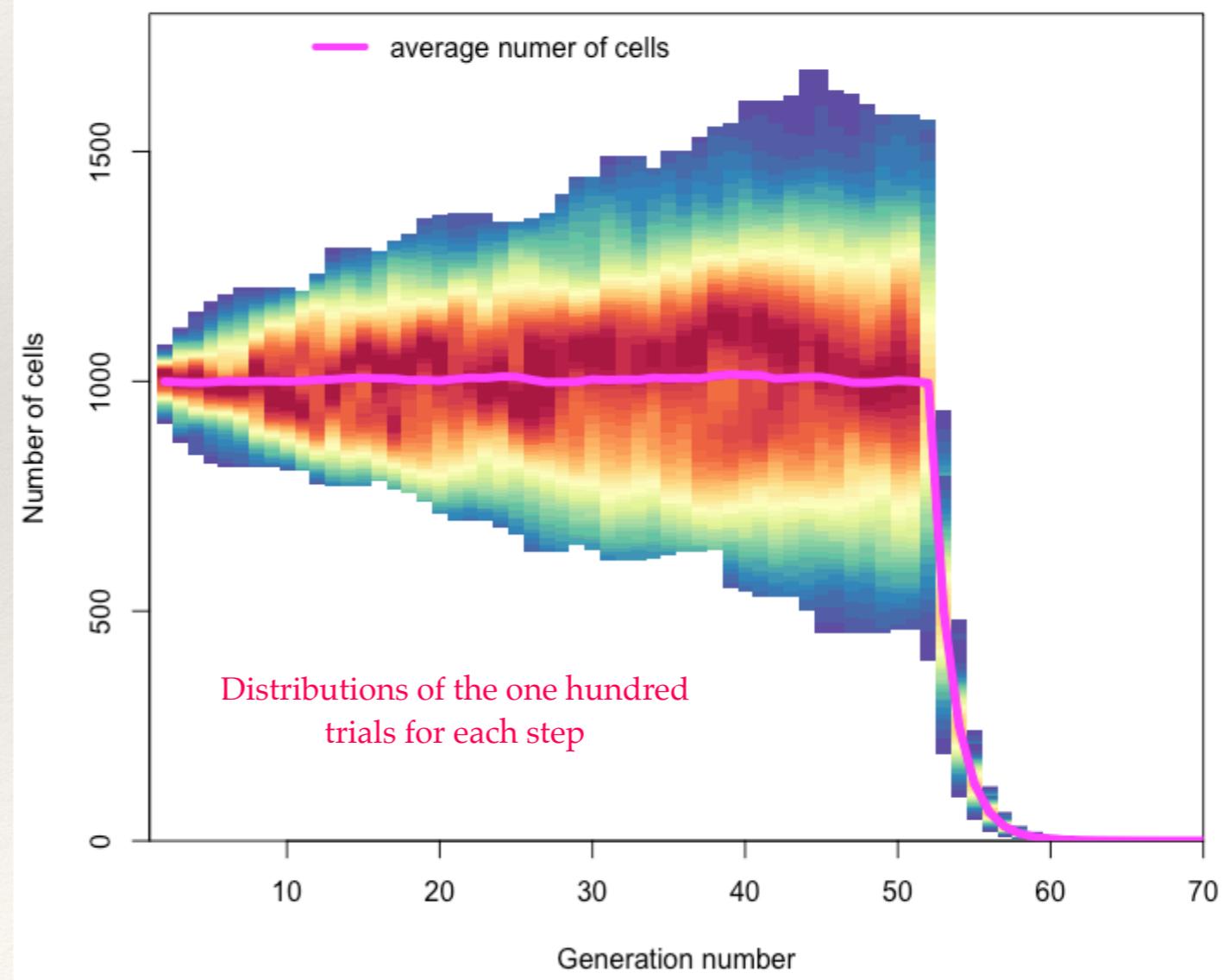
One hundred trials



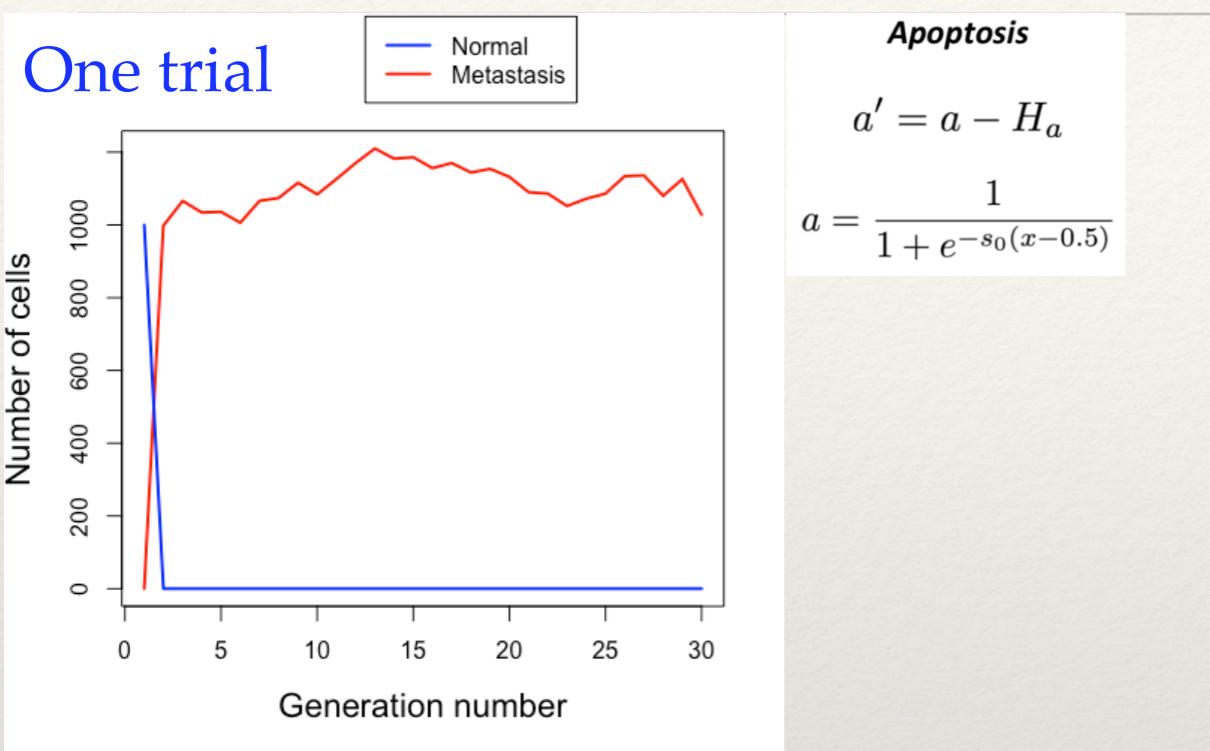
$$\begin{aligned} & \text{Apoptosis} \\ & a' = a - H_a \\ & a = \frac{1}{1 + e^{-s_0(x-0.5)}} \end{aligned}$$

d=1. a=0.5 (in the code)
without the dependence on a mutation density

One hundred trials

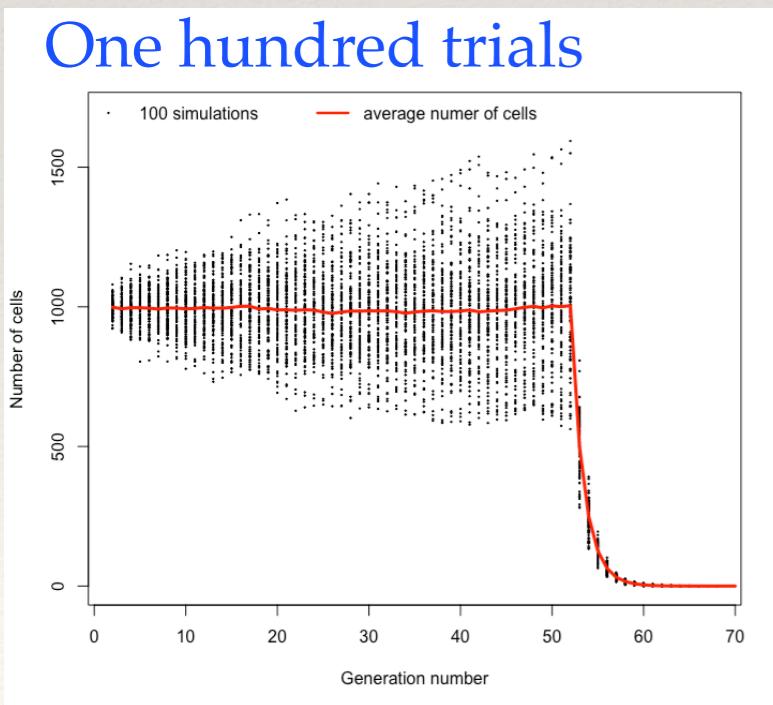
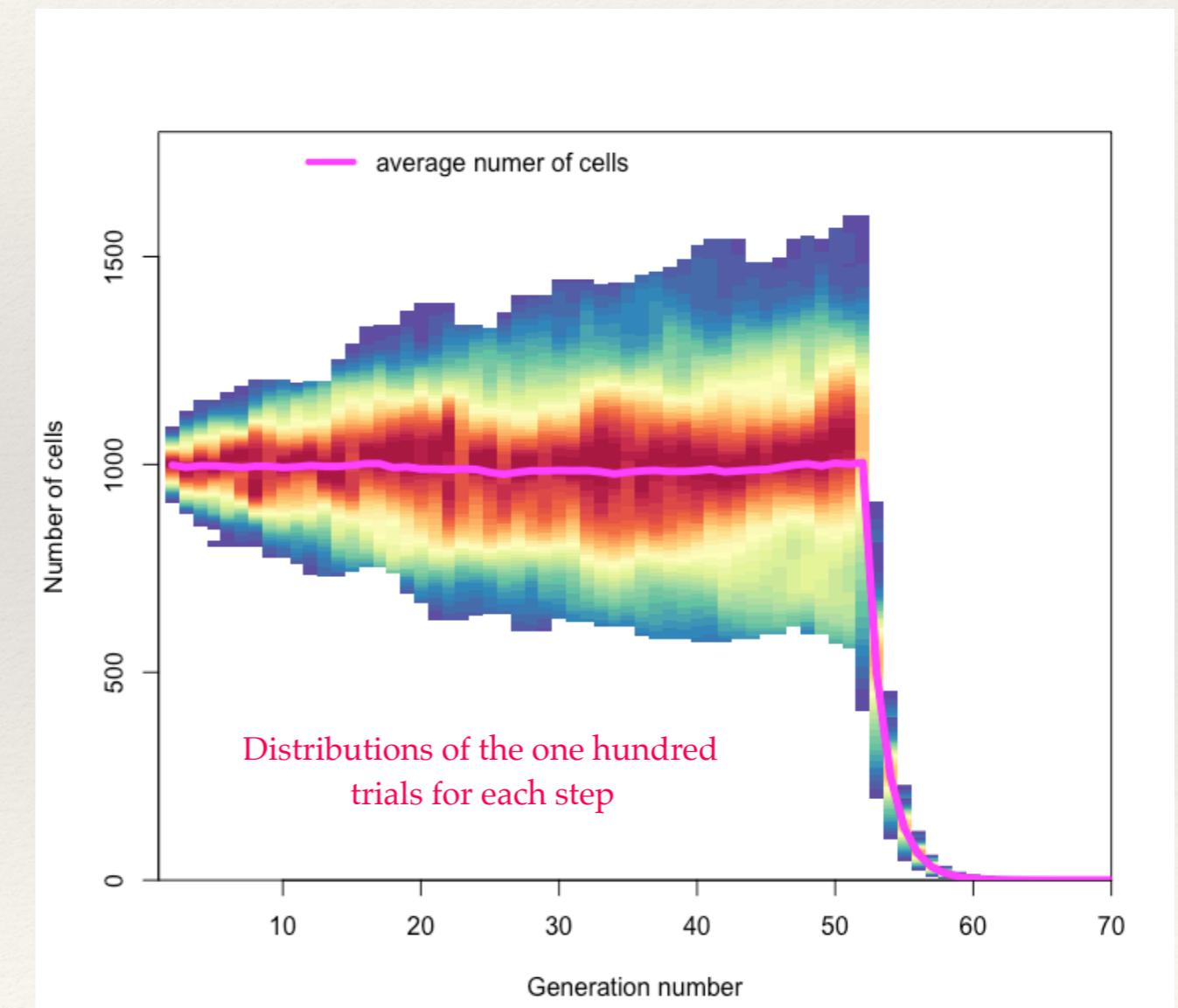


Test 3: Apoptosis death: Metastasis cells

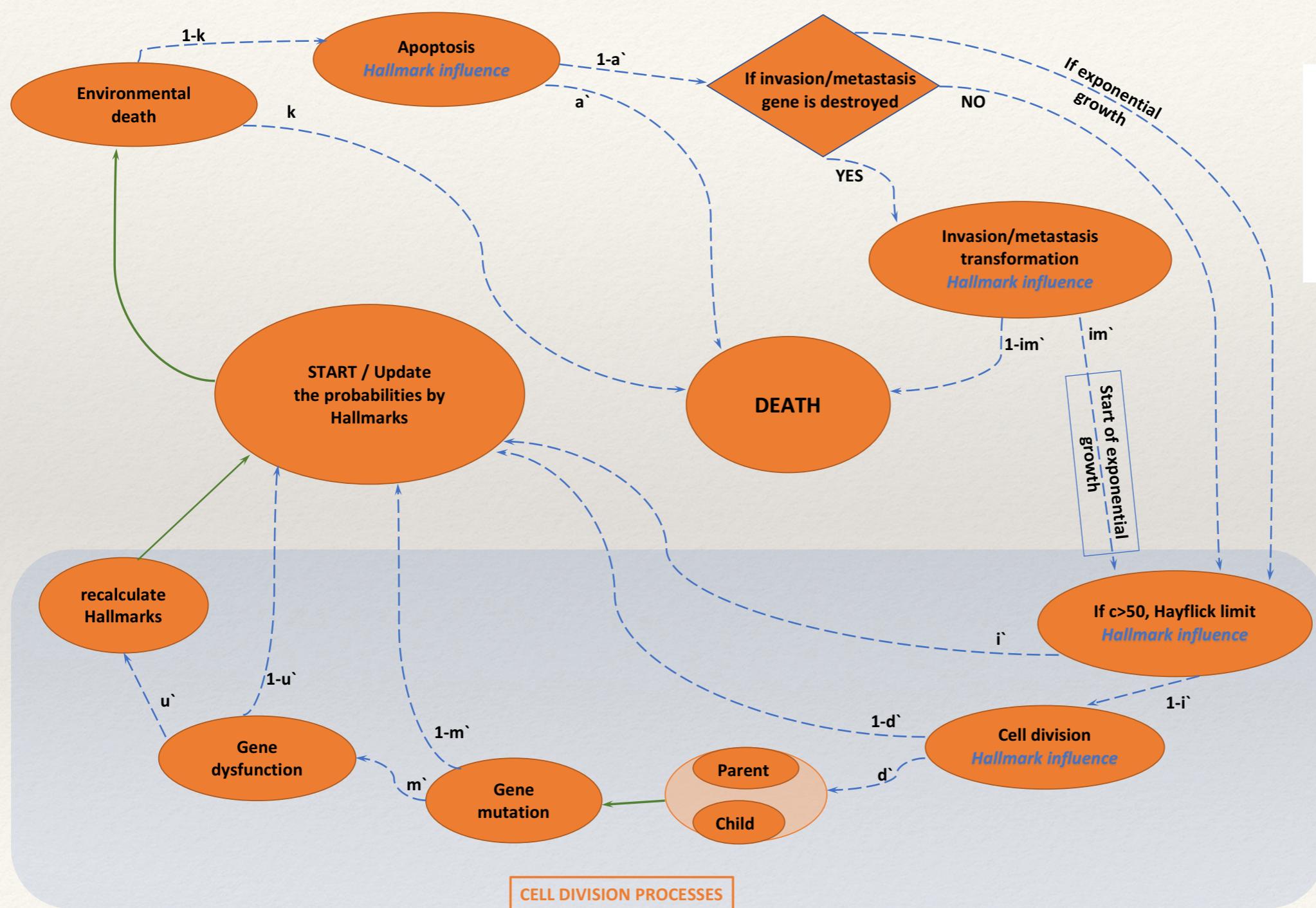


$d=1.$ $a=0.5$ (in the code)
without the dependence on a mutation density

One hundred trials



Test 4: Invasion/metastasis transformation: the results of simulation



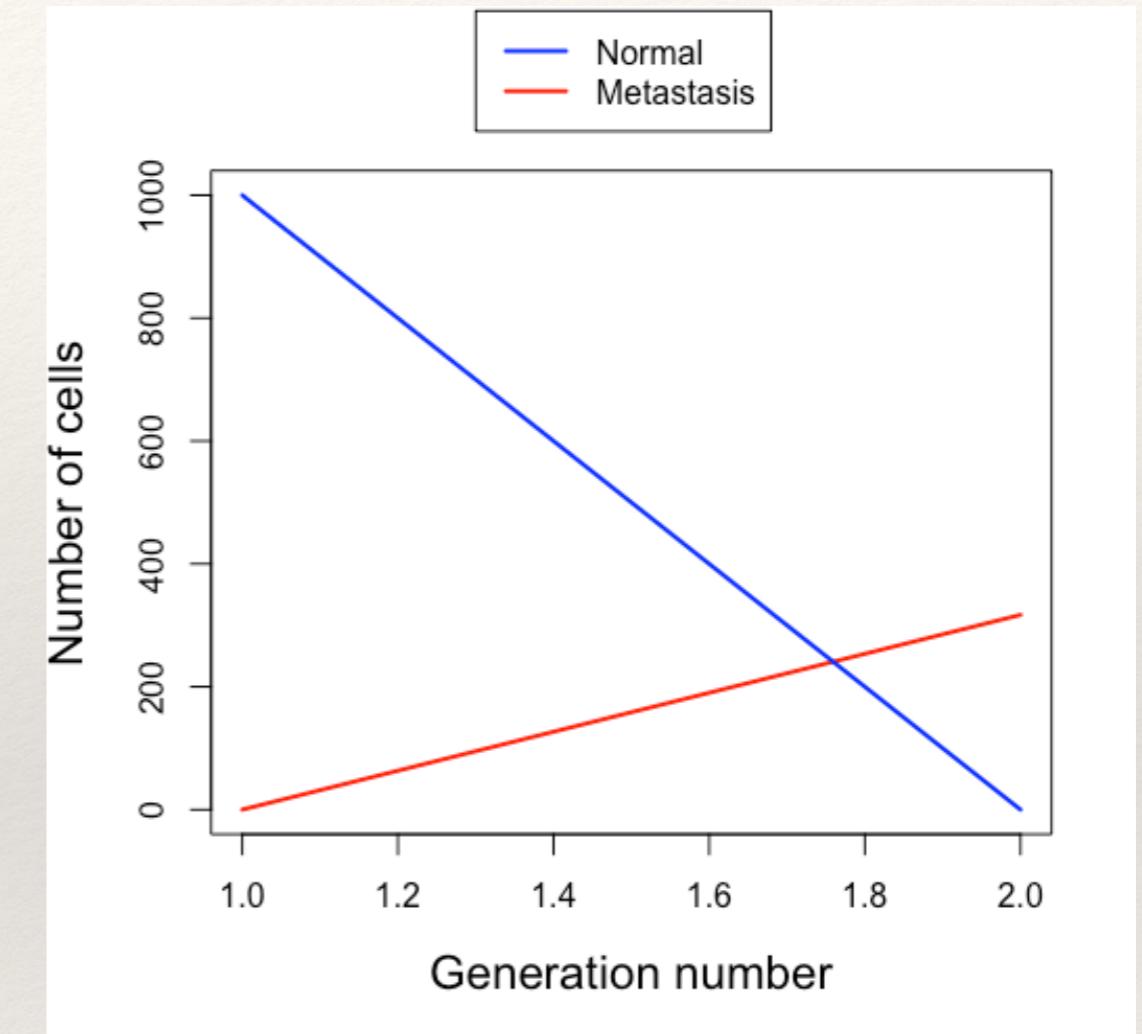
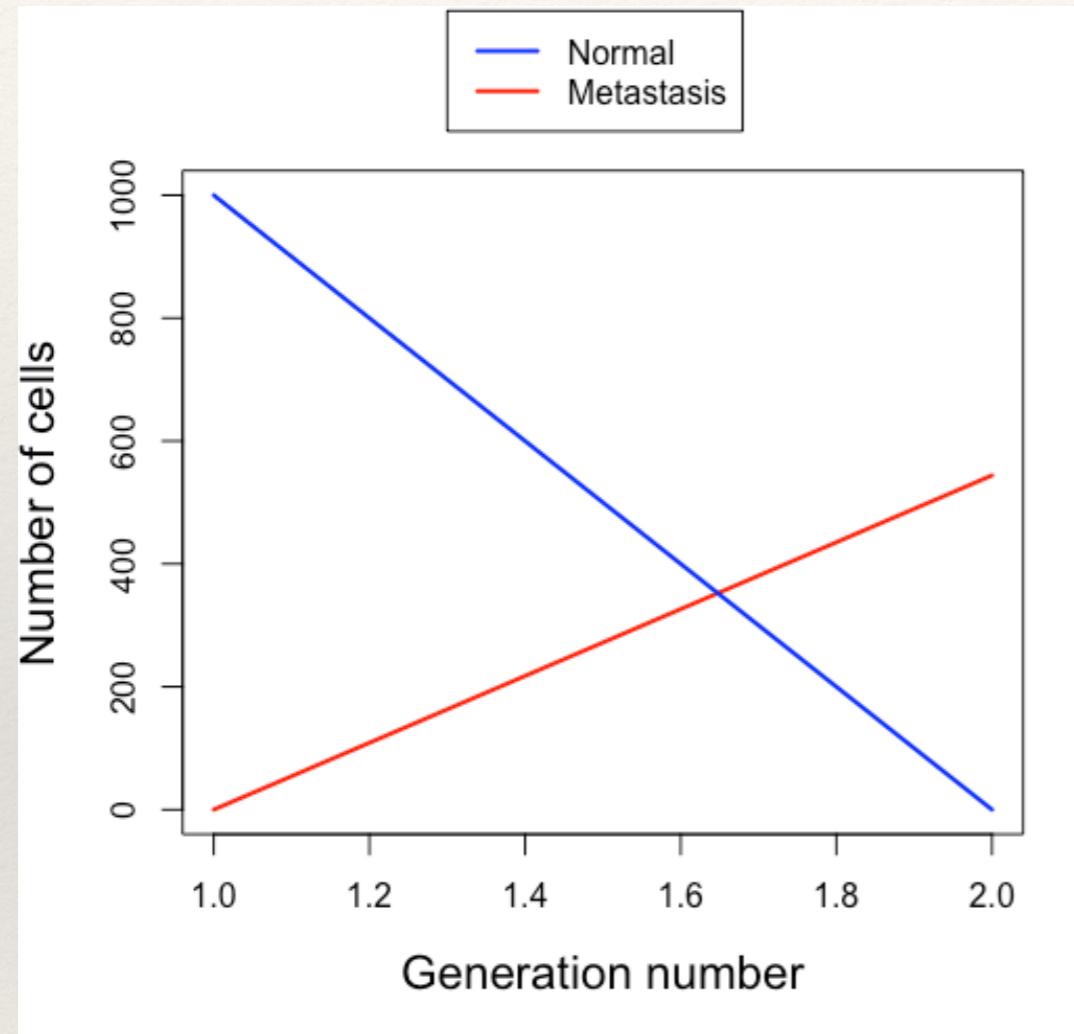
Invasion/metastasis transformation

$$im' = H_{im}$$

$$d=0$$

$$a'=0 \text{ (in the code)}$$

Test 4: Invasion/metastasis transformation: the results of simulation



$$d=0, \quad a' = 0$$

$$im=0.5 \text{ and}$$

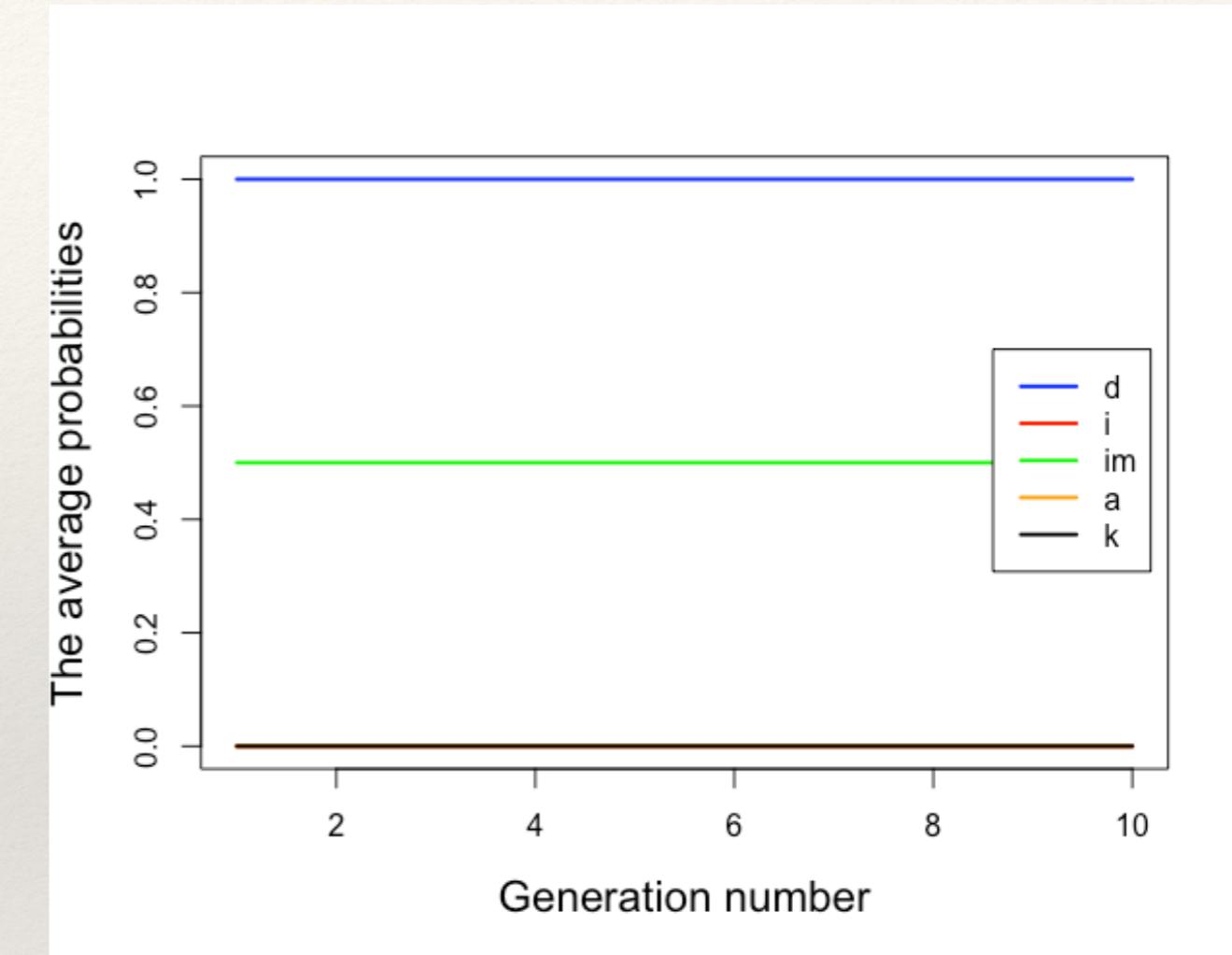
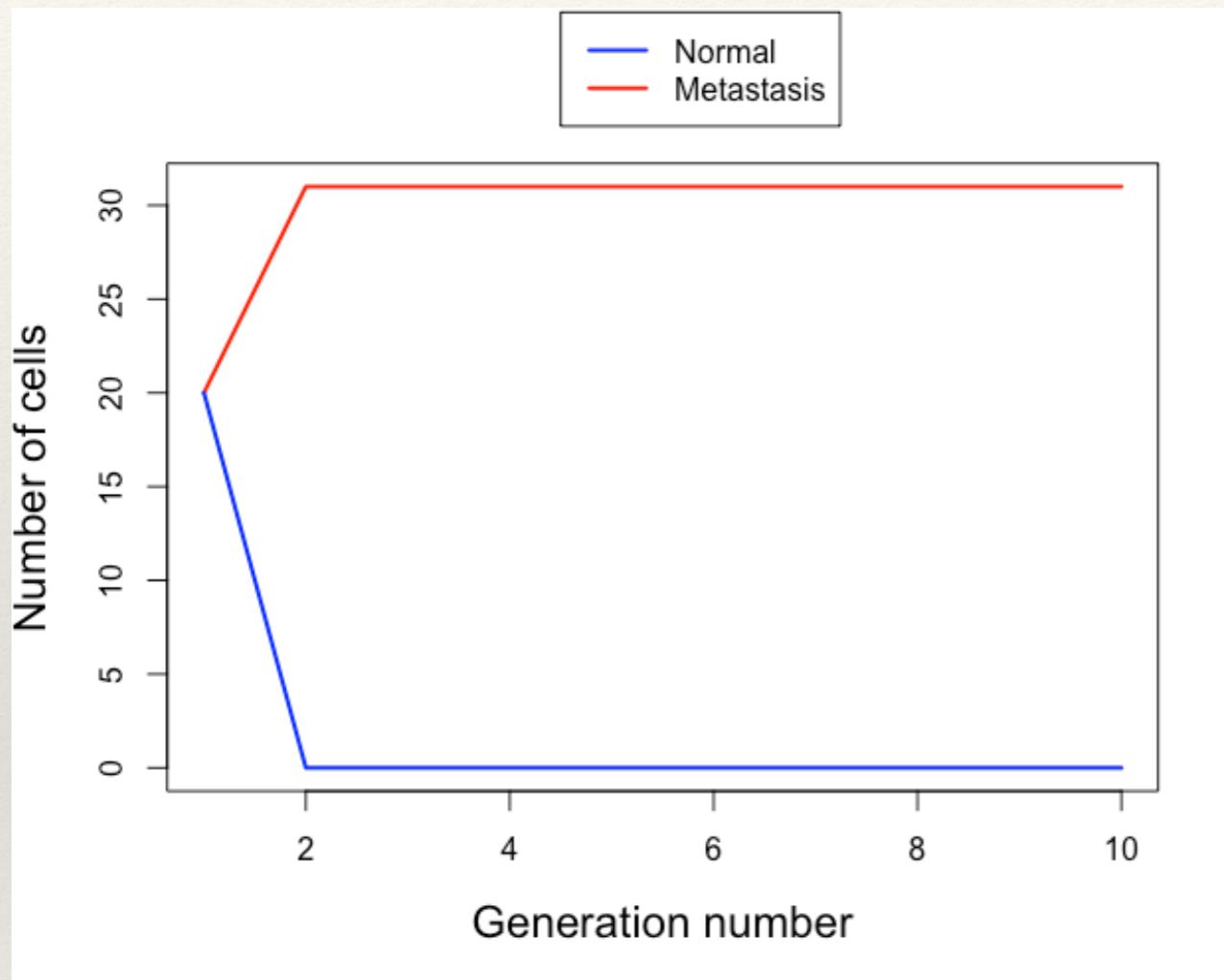
the transformation of half of cells

$$d=0, \quad a' = 0$$

$$im=0.3 \text{ and}$$

the transformation of 0.3 of all cells

Test 4: Invasion/metastasis transformation: the results of simulation



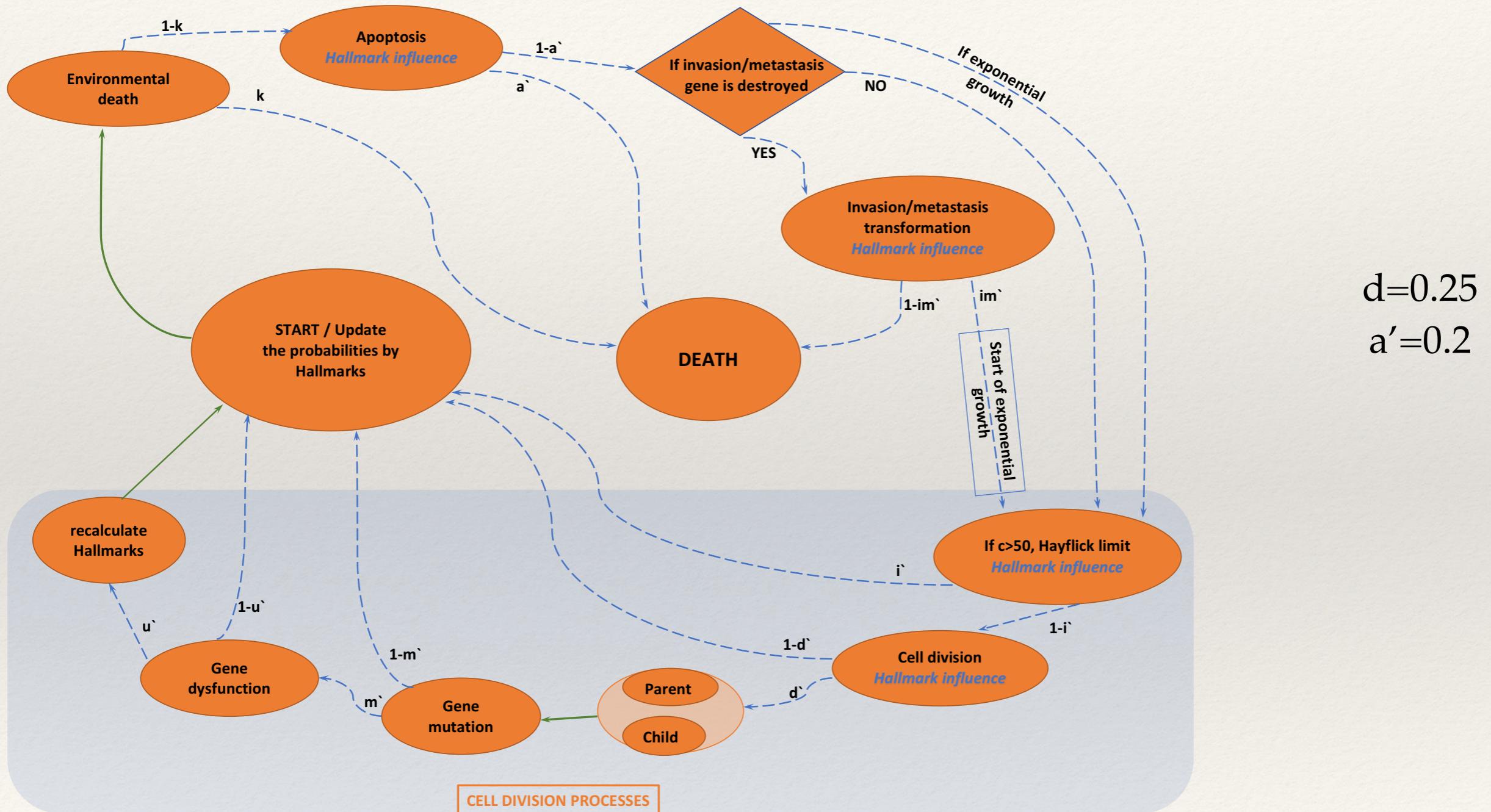
**Invasion/metastasis
transformation**

$$im' = H_{im}$$

$$d=0, a=0 \\ im=0.5$$

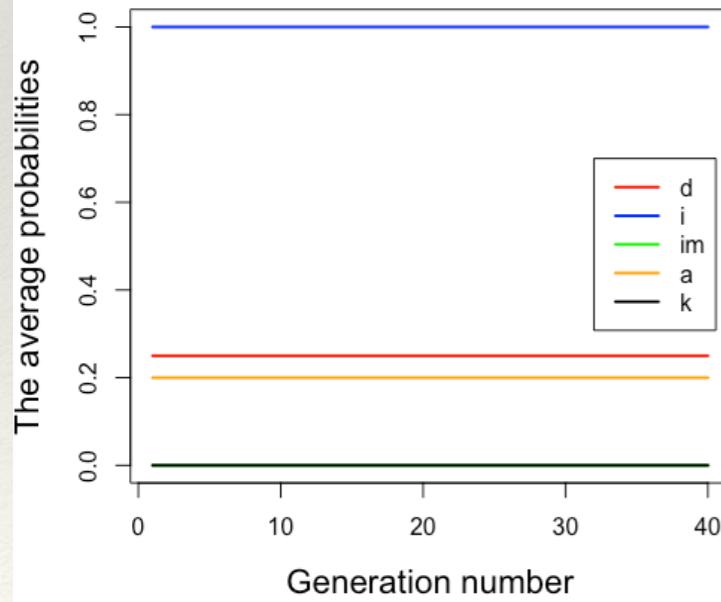
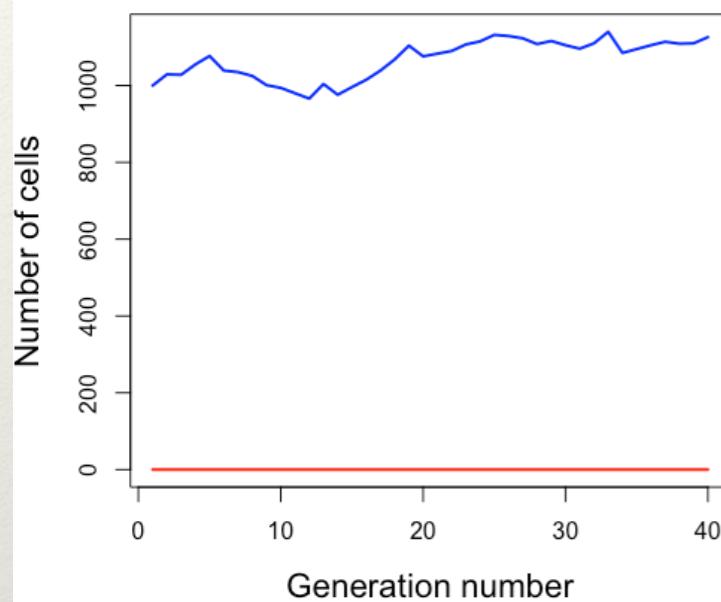
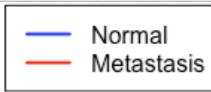
mixed initial cells: normal and metastasis

Test 5: Cell division process: the results of simulation



Test 5: Cell division process: Normal cells

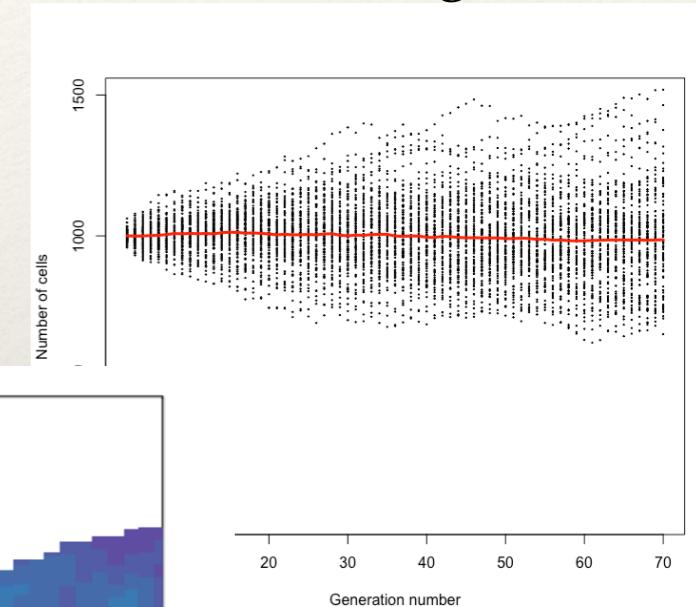
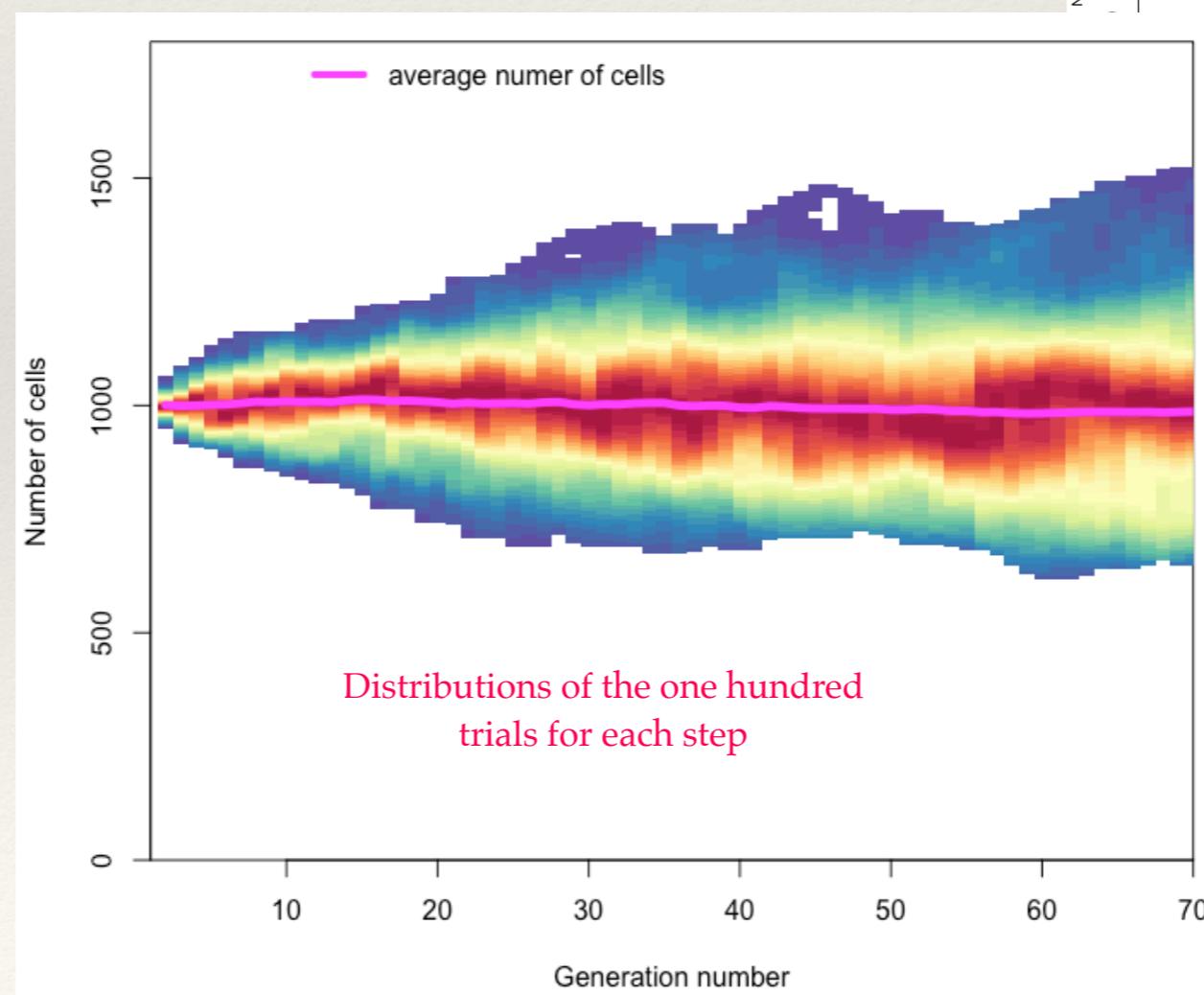
One trial



$$d=0.25. \quad a'=0.2. \quad \text{Because } 0.8 * 1.25 = 1$$

It allows us to keep the number of cells in average

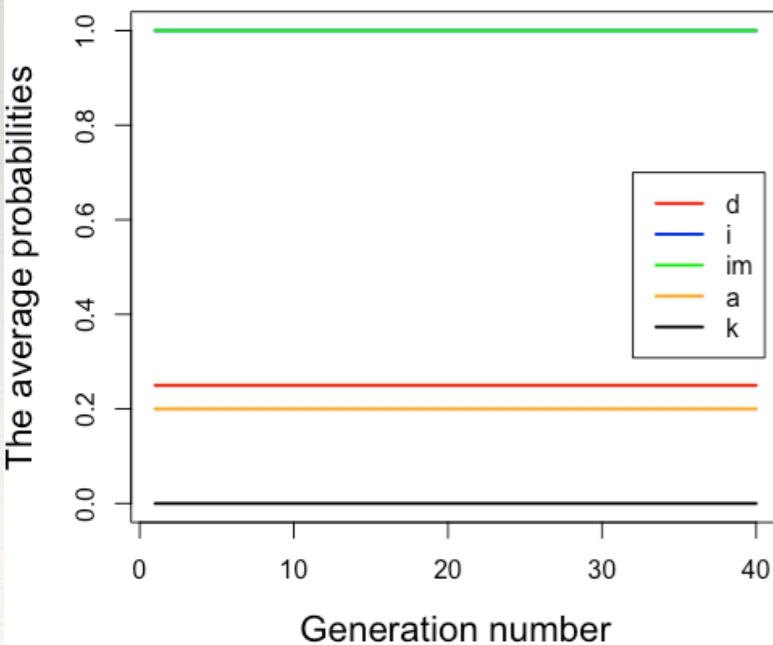
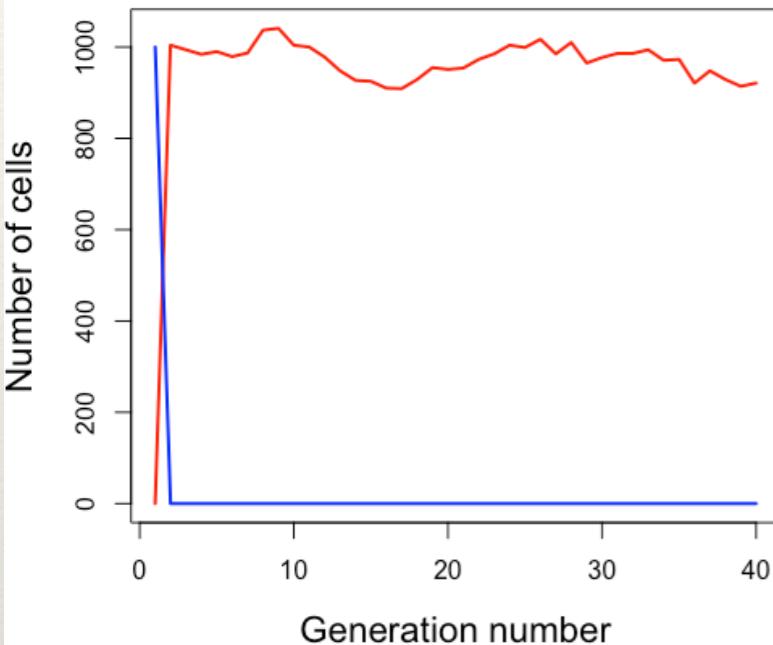
One hundred trials



Test 5: Cell division process: Metastasis cells

One trial

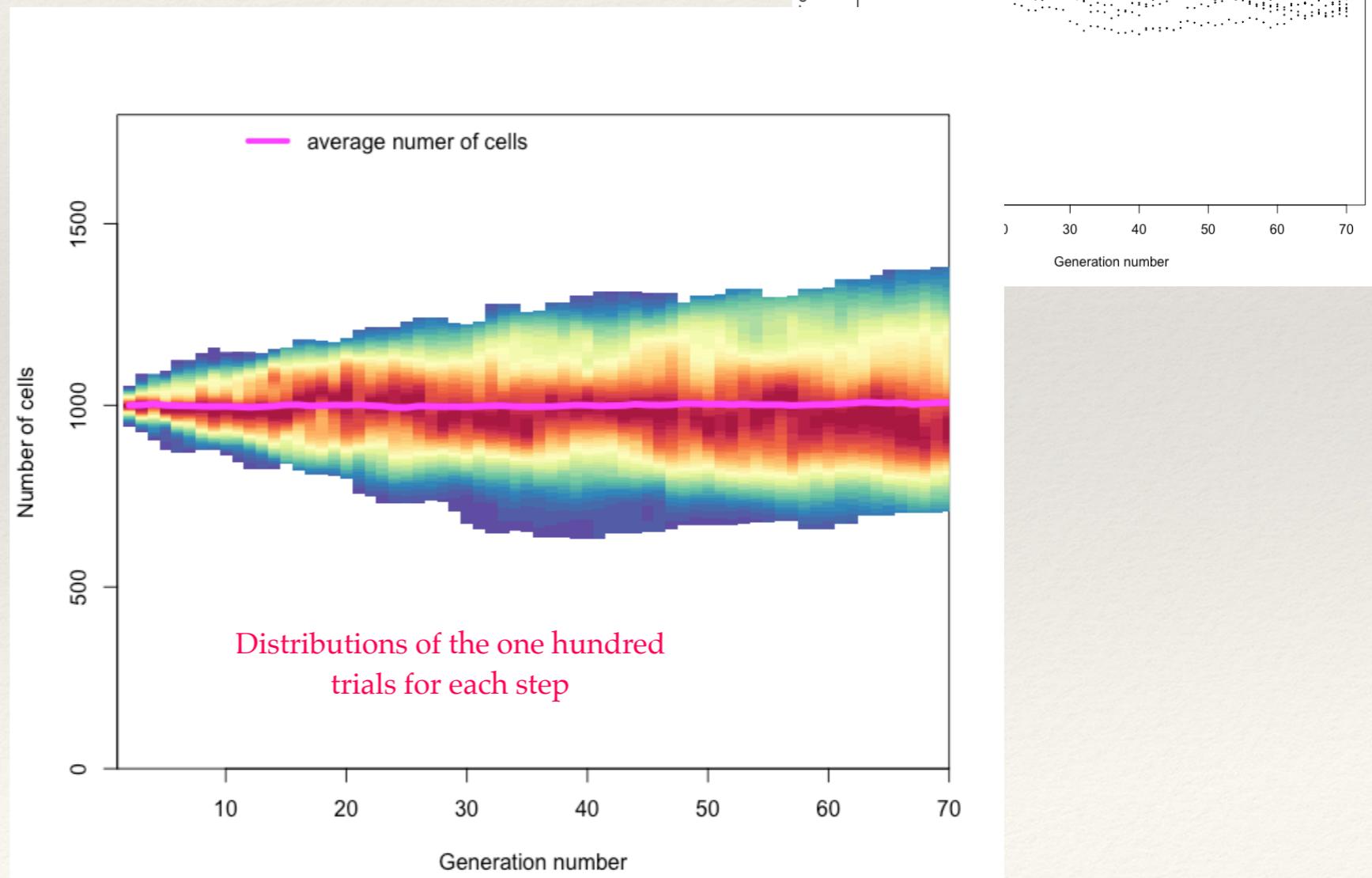
Normal
Metastasis



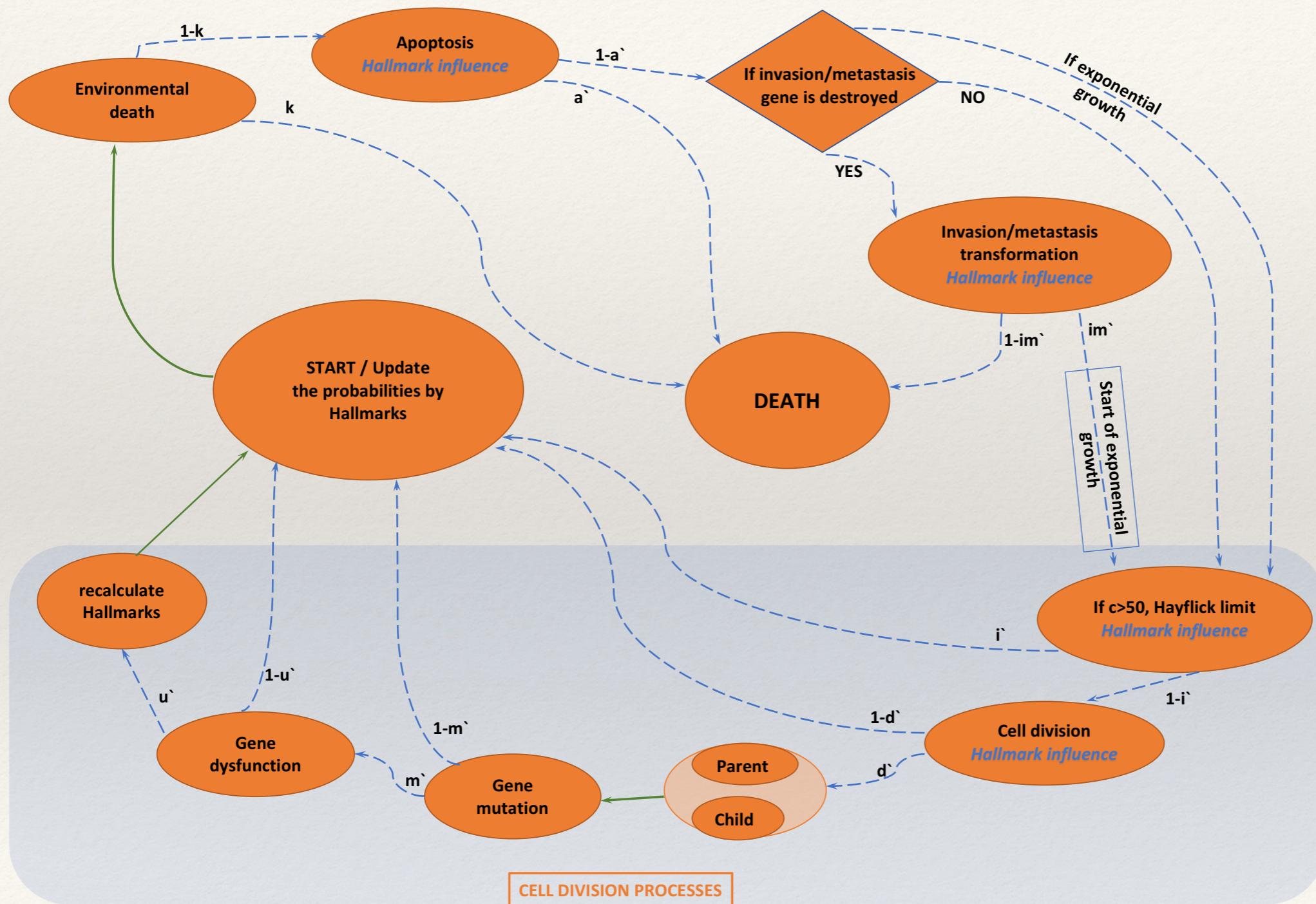
$$d=0.25. \quad a'=0.2. \quad \text{Because } 0.8 * 1.25 = 1$$

It allows us to keep the number of cells in average

One hundred trials



Test 6: Heyflick limit without Hallmarks influence

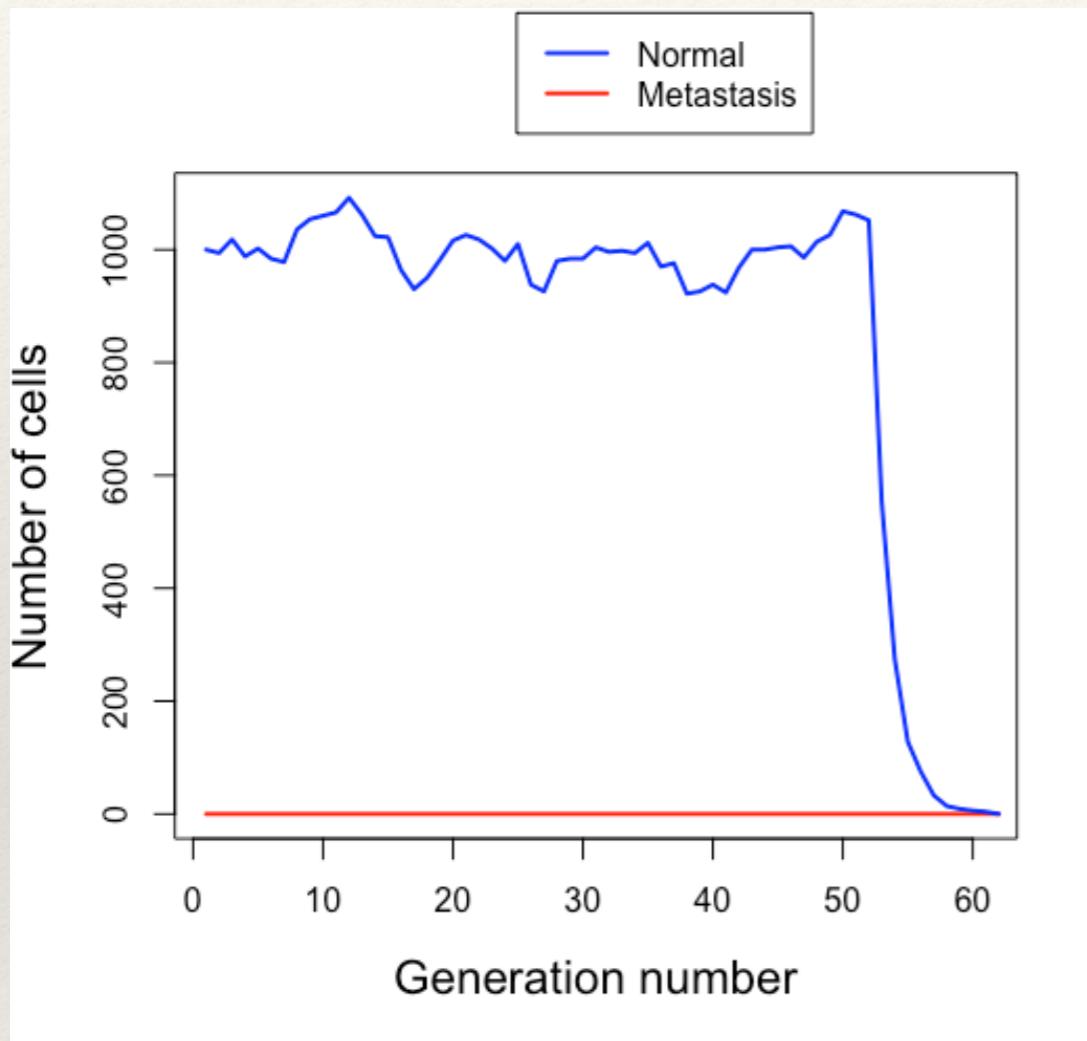


$d=1$

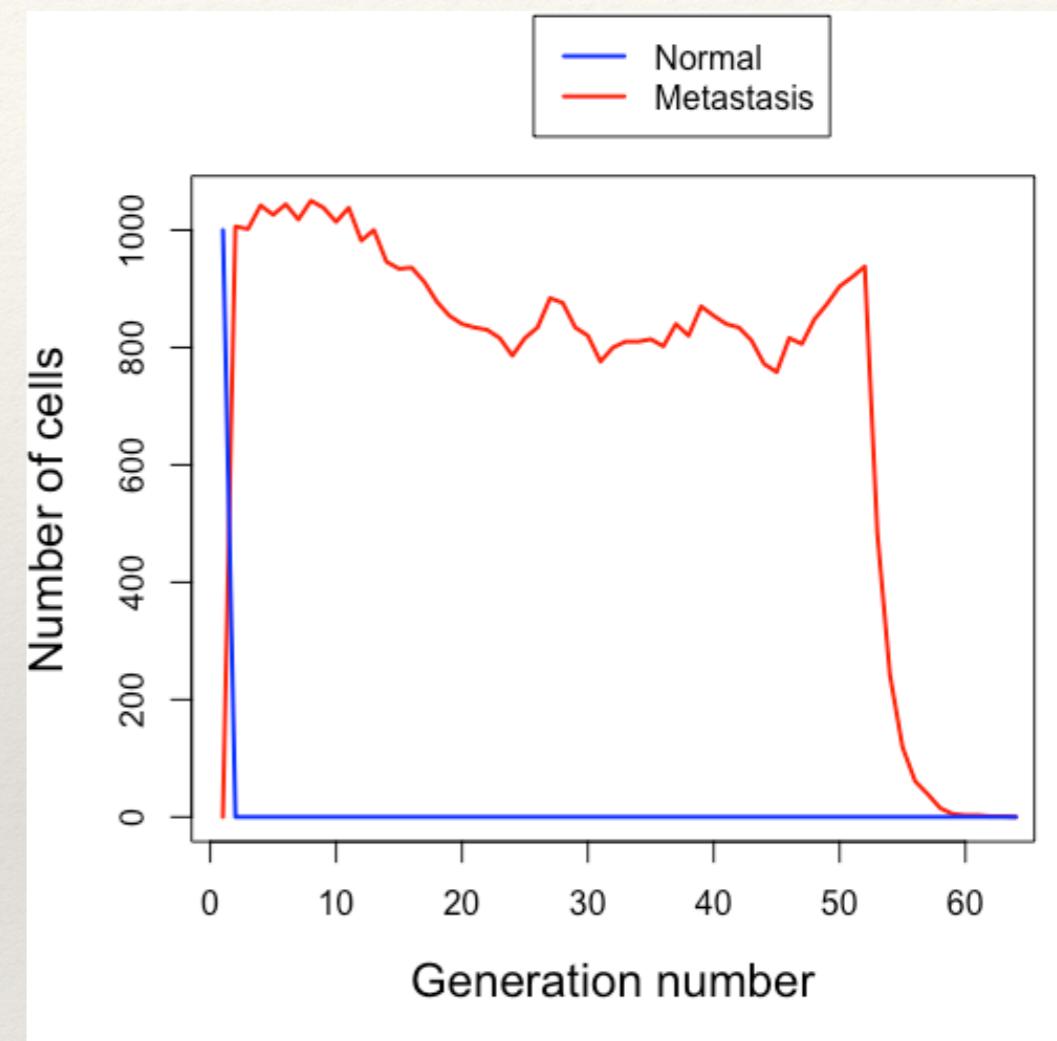
$c_{max}=0$

$i=0.5$ (in the code)

Test 6: Heyflick limit: the results of simulation



$N=1000$
 $d=1$ and $k=0.5$
 $c_{\max}=50$



The average for 100 simulation is shown at the Test N 2

The Hallmarks tests: gene file

Name	Length of CDS	Hallmark type	Oncogene or suppressor	weights
GA1	1	apoptosis	o	0.1
GA2	1	apoptosis	o	0.2
GA3	1	apoptosis	o	0.3
GA4	1	apoptosis	o	0.4
GB1	1	angiogenesis	o	0.1
GB2	1	angiogenesis	o	0.2
GB3	1	angiogenesis	o	0.3
GB4	1	angiogenesis	o	0.4
GIM1	1	invasion	o	0.1
GIM2	1	invasion	o	0.2
GIM3	1	invasion	o	0.3
GIM4	1	invasion	o	0.4
GI1	1	immortalization	o	0.1
GI2	1	immortalization	o	0.2
GI3	1	immortalization	o	0.3
GI4	1	immortalization	o	0.4
GD1	1	growth	o	0.1
GD2	1	anti-growth	o	0.2
GD3	1	growth	o	0.3
GD4	1	anti-growth	o	0.4



The Hallmarks tests: cell init file

N	Destroyed Genes	Calculation example	Notes
1	GB1, GB2, GB3, GB4, GI1, GI2, GI3, GI4, GD1, GD2, GD3, GD4	a=0.73..., Ha=0	For normal cells a=sigma function - Ha, the mutation rates are same for different cells and rate = 12/20, s0=10 and sigma=function = 0.73...
2	GA1, GB1, GB2, GB3, GB4, GI1, GI2, GI3, GI4, GD1, GD2, GD3	a=0.63..., Ha=0.1	
3	GA1, GA2, GB1, GB2, GB3, GB4, GI1, GI2, GI3, GI4, GD1, GD2	a=0.43..., Ha=0.3	
4	GA1, GA2, GA3, GB1, GB2, GB3, GB4, GI1, GI2, GI3, GI4, GD1	a=0.13..., Ha=0.6	
5	GA1, GA2, GA3, GA4, GB1, GB2, GB3, GB4, GI1, GI2, GI3, GI4	a=0, Ha=1	
6	GIM1, GA1, GA2, GA3, GA4, GB1, GB2, GB3, GB4, GI1, GI2, GI3	a=0, Ha=1	For metastasis cells a=sigma function - Ha, Values are same as for normal cells
7	GIM1, GA1, GA2, GA3, GB1, GB2, GB3, GB4, GI1, GI2, GI3, GI4	a=0.13..., Ha=0.6	
8	GIM1, GA1, GA2, GB1, GB2, GB3, GB4, GI1, GI2, GI3, GI4, GD1	a=0.43..., Ha=0.3	
9	GIM1, GA1, GB1, GB2, GB3, GB4, GI1, GI2, GI3, GI4, GD1, GD2	a=0.63..., Ha=0.1	
10			Normal cell

Apoptosis

$$a' = a - H_a$$

$$a = \frac{1}{1 + e^{-s_0(x-0.5)}}$$

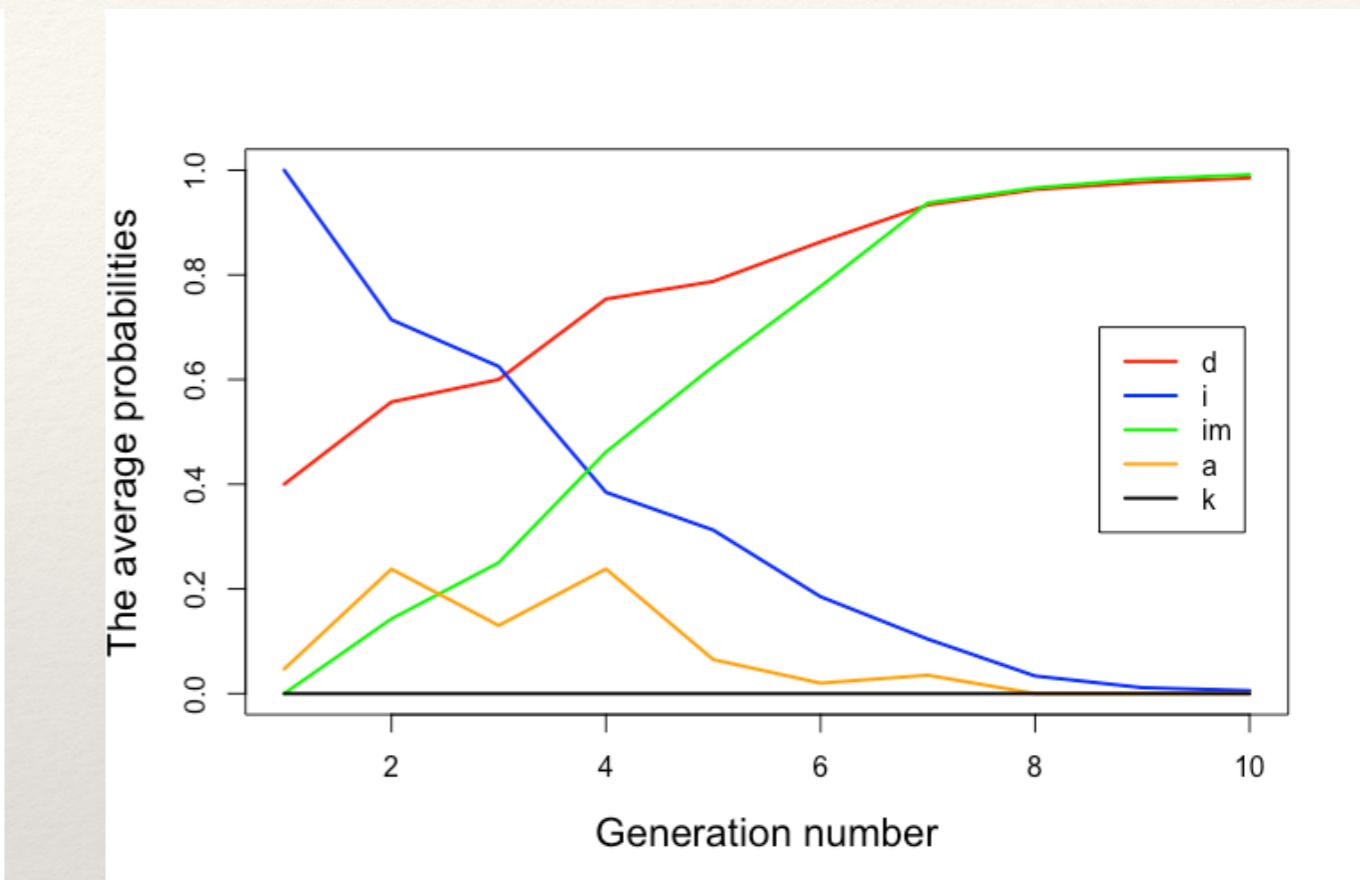
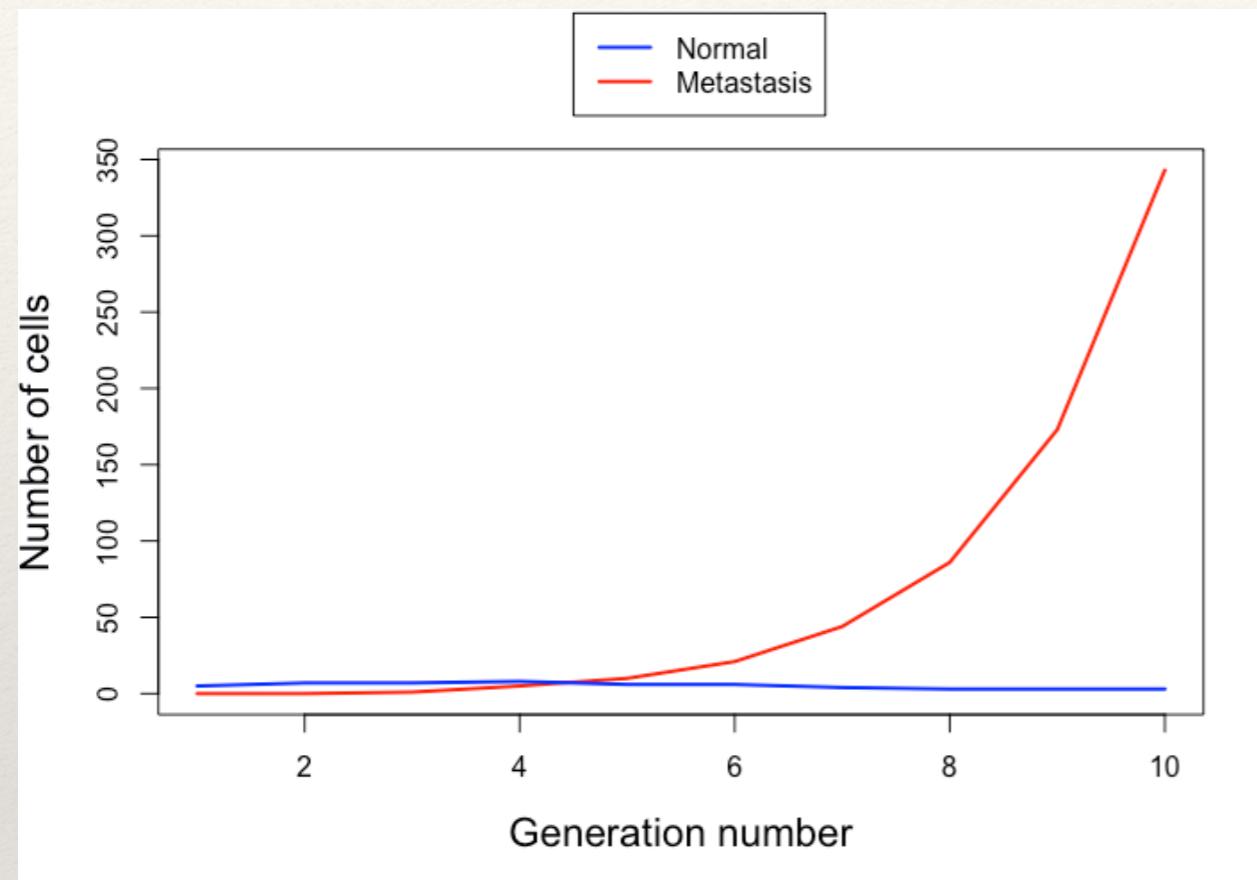
The Hallmarks tests: cell init file

11	GB1, <u>GD1, GD2, GD3, GD4</u>	$H_b=0.1, N_{max}=24,$ $d=0.083\dots$	$d' = \begin{cases} d - E' \times N, & \text{when logistic growth} \\ d, & \text{when exponential growth} \end{cases}$ where $d = d_0 + H_d$, what is why we have $H_d=1$ with 4 genes (GD1-GD4)
12	GB1, <u>GB2, GD1, GD2, GD3, GD4</u>	$H_b=0.3, N_{max}=32,$ $d=0.3125$	
13	GB1, <u>GB2, GB3, GD1, GD2, GD3, GD4</u>	$H_b=0.6, N_{max}=44, d=0.5$	
14	GB1, <u>GB2, GB3, GB4, GD1, GD2, GD3, GD4</u>	$H_b=1.0, N_{max}=60,$ $d=0.633\dots$	
15	GIM2, <u>GB1, GB2, GB3, GB4, GD1, GD2, GD3, GD4</u>	$H_b=1.0, N_{max}=60, d=1$	$N_{max} = \frac{1 + F_0 \cdot H_b}{E_0}$ $E' = \frac{E_0}{1 + F_0 \cdot H_b}$ $F_0=2, E_0=0.05, \text{ so } N_{max}=(20-60) \text{ to check formula,}$ $N=22$
16	GIM2, <u>GB1, GB2, GB3, GD1, GD2, GD3, GD4</u>	$H_b=0.6, N_{max}=32, d=1$	
17	GIM2, <u>GB1, GB2, GD1, GD2, GD3, GD4</u>	$H_b=0.3, N_{max}=44, d=1$	
18	GIM2, <u>GB1, GD1, GD2, GD3, GD4</u>	$H_b=0.1, N_{max}=24, d=1$	
19			Normal cell
20	GIM1	$H_{im}=0.1, i_m'=0.1 \text{ and } 1$	This is a invasion/metastasis transformation, the probability i_m' = H_{im} before transformation and i_m' = 1 after transformation
21	GIM1, <u>GIM2</u>	$H_{im}=0.3, i_m'=0.3 \text{ and } 1$	
22	GIM1, <u>GIM2, GIM3</u>	$H_{im}=0.6, i_m'=0.6 \text{ and } 1$	
23	GIM1, <u>GIM2, GIM3, GIM4</u>	$H_{im}=1.0, i_m'=1.0 \text{ and } 1$	
24			Normal cell
25	GI1	$H_i=0.1, i'=0.9$	$i' = 1 - H_i$
26	GI1, <u>GI2</u>	$H_i=0.3, i'=0.7$	
27	GI1, <u>GI2, GI3</u>	$H_i=0.6, i'=0.4$	
28	GI1, <u>GI2, GI3, GI4</u>	$H_i=1.0, i'=0$	
29	GIM3, <u>GI1, GI2, GI3, GI4</u>	$H_i=1.0, i'=0$	
30	GIM3, <u>GI1, GI2, GI3</u>	$H_i=0.6, i'=0.4$	
31	GIM3, <u>GI1, GI2</u>	$H_i=0.3, i'=0.7$	
32	GIM3, <u>GI1</u>	$H_i=0.1, i'=0.9$	Same formula
33			Normal cell
34	GD1	$H_d=0.1, d'=0$	$d' = \begin{cases} d - E' \times N, & \text{when logistic growth} \\ d, & \text{when exponential growth} \end{cases}$ where $d = d_0 + H_d$, $d_0=0, N/N_{max}=22/20 > 1$, $\text{so } d=0.$
35	GD1, <u>GD2</u>	$H_d=0.3, d'=0$	
36	GD1, <u>GD2, GD3</u>	$H_d=0.6, d'=0$	
37	GD1, <u>GD2, GD3, GD4</u>	$H_d=1.0, d'=0$	
38	GIM4, <u>GD1, GD2, GD3, GD4</u>	$H_d=1.0, d'=1.0$	
39	GIM4, <u>GD1, GD2, GD3</u>	$H_d=0.6, d'=0.6$	$d=d_0+H_d, d_0=0$
40	GIM4, <u>GD1, GD2</u>	$H_d=0.3, d'=0.3$	
41	GIM4, <u>GD1</u>	$H_d=0.1, d'=0.1$	
42			Normal cell

The Hallmarks tests: cell out file

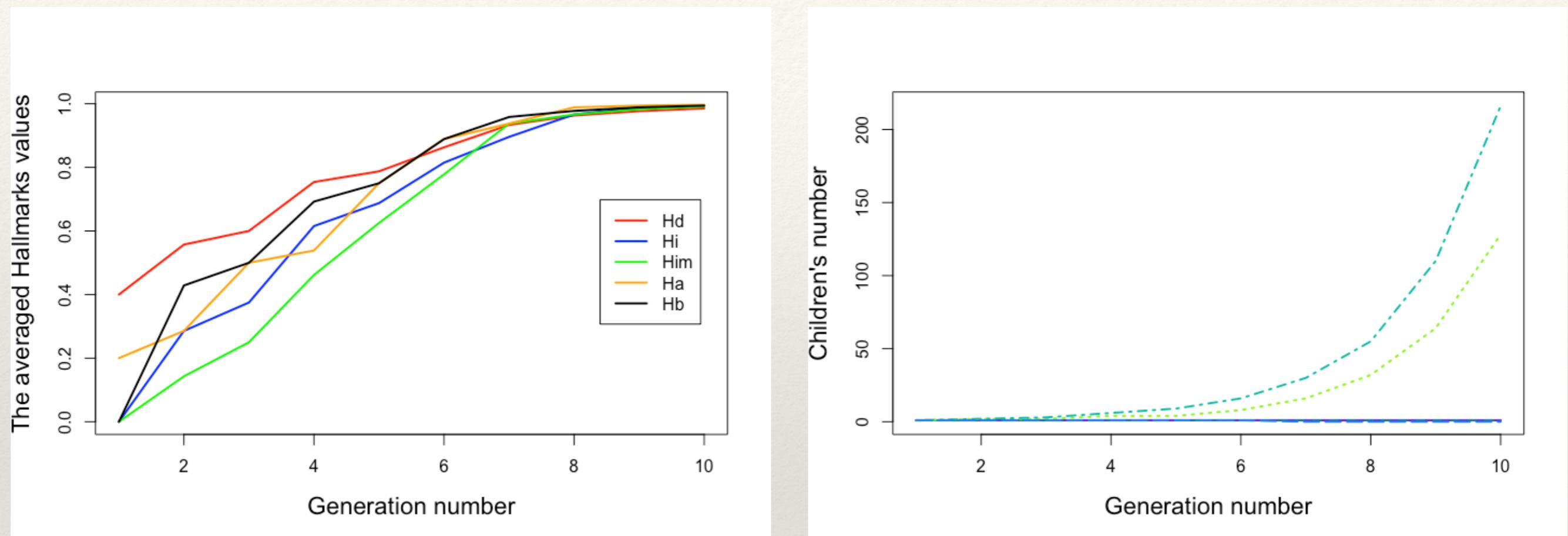
1	20	20	0	0	0	1	0.1	0.01098694	0	0.05	42	20	0	0	0.1	0	0	0	0	0	Him TEST	Please, see the step 2 to compare with metastasis cells					
1	21	21	0	0	0	1	0.3	0.01798621	0	0.05	42	20	0	0	0.3	0	0	0	0	0							
1	22	22	0	0	0	1	0.6	0.02931223	0	0.05	42	20	0	0	0.6	0	0	0	0	0							
1	23	23	0	0	0	1	1	0.04742587	0	0.05	42	20	0	0	1	0	0	0	0	0							
Time	AvgOrIndx	ID	ParentID	c'	d'	i'	im'	a'	k'	E'	N	Nmax'	M	Ha	Him	Hi	Hd	Hb	type	PosDriver:G	PosDriver:G	PosDriver:G	PosDriver:G	PosDriver:G	PosDriver:G		
2	1	1	0	0	0.63333333	0	0	0.73105858	0	0.01666667	22	60	20	0	0	1	1	1	0	Apoptosis test						1	
2	2	2	0	0	0.23333333	0	0	0.63105858	0	0.01666667	22	60	20	0.1	0	1	0.6	1	0						1	1	
2	3	3	0	0	0	0	0	0.43105858	0	0.01666667	22	60	20	0.3	0	1	0.3	1	0						1	1	
2	4	4	0	0	0	0	0	0.13105858	0	0.01666667	22	60	20	0.6	0	1	0.1	1	0						1	1	
2	5	5	0	0	0	0	0	0	0	0.01666667	22	60	20	1	0	1	0	1	0						1	1	
2	6	6	0	0	0	0.4	1	0	0	0.01666667	22	60	20	1	0.1	0.6	0	1	1						1	1	
2	7	7	0	0	0	0	0	1	0.13105858	0	0.01666667	22	60	20	0.6	0.1	1	0	1	1						1	1
2	8	8	0	0	0.1	0	0	1	0.43105858	0	0.01666667	22	60	20	0.3	0.1	1	0.1	1	1						1	1
2	9	9	0	0	0.3	0	0	1	0.63105858	0	0.01666667	22	60	20	0.1	0.1	0.3	1	1	1						1	1
2	10	10	0	0	1	0	0.00669285	0	0.05	22	20	20	0	0	0	0	0	0	0	Normal cell							
2	11	11	0	0	0.08333333	1	0	0.07585818	0	0.04166667	22	24	20	0	0	0	1	0.1	0	Hb TEST							1
2	12	12	0	0	0.3125	1	0	0.11920292	0	0.03125	22	32	20	0	0	0	0	1	0.3							1	1
2	13	13	0	0	0.5	1	0	0.18242552	0	0.02272727	22	44	20	0	0	0	0	1	0.6							1	1
2	14	14	0	0	0.63333333	1	0	0.26894142	0	0.01666667	22	60	20	0	0	0	0	1	1							1	1
2	15	15	0	0	1	1	1	1	0.37754067	0	0.01666667	22	60	20	0.2	0	1	1	1							1	1
2	16	16	0	0	1	1	1	1	0.26894142	0	0.02272727	22	44	20	0	0.2	0	1	0.6							1	1
2	17	17	0	0	1	1	1	1	0.18242552	0	0.03125	22	32	20	0	0.2	0	1	0.3							1	1
2	18	18	0	0	1	1	1	1	0.11920292	0	0.04166667	22	24	20	0	0.2	0	1	0.1							1	1
2	19	19	0	0	1	0	0.00669285	0	0.05	22	20	20	0	0	0	0	0	0	0	Normal cell							
2	20	20	0	0	0	1	1	0.01098694	0	0.05	22	20	20	0	0.1	0	0	0	1	Him TEST	Please, see the step 1 to compare with normal cells						
2	21	21	0	0	0	1	1	0.01798621	0	0.05	22	20	20	0	0.3	0	0	0	1								
2	22	22	0	0	0	1	1	0.02931223	0	0.05	22	20	20	0	0.6	0	0	0	1								
2	23	23	0	0	0	1	1	0.04742587	0	0.05	22	20	20	0	1	0	0	0	1								
2	24	24	0	0	1	0	0.00669285	0	0.05	22	20	20	0	0	0	0	0	0	0	Normal cell							
2	25	25	0	0	0.9	0	0	0.01098694	0	0.05	22	20	20	0	0	0.1	0	0	0	H1 TEST							
2	26	26	0	0	0.7	0	0	0.01798621	0	0.05	22	20	20	0	0	0.3	0	0	0								
2	27	27	0	0	0.4	0	0	0.02931223	0	0.05	22	20	20	0	0	0.6	0	0	0								
2	28	28	0	0	0	0	0	0.04742587	0	0.05	22	20	20	0	0	1	0	0	0								
2	29	29	0	0	0	0	1	0.07585818	0	0.05	22	20	20	0	0.3	1	0	0	1								
2	30	30	0	0	0	0	0	1	0.04742587	0	0.05	22	20	20	0	0.3	0.6	0	0								
2	31	31	0	0	0	0	0	1	0.02931223	0	0.05	22	20	20	0	0.3	0.3	0	0								
2	32	32	0	0	0	0	0	1	0.01798621	0	0.05	22	20	20	0	0.3	0.1	0	0								
2	33	33	0	0	1	0	0.00669285	0	0.05	22	20	20	0	0	0	0	0	0	0	Normal cell							
2	34	34	0	0	0	1	0	0.01098694	0	0.05	22	20	20	0	0	0	0	0.1	0	Hd TEST							
2	35	35	0	0	0	1	0	0.01798621	0	0.05	22	20	20	0	0	0	0	0.3	0								
2	36	36	0	0	0	1	0	0.02931223	0	0.05	22	20	20	0	0	0	0	0.6	0								
2	37	37	0	0	0	1	0	0.04742587	0	0.05	22	20	20	0	0	0	0	1	0								
2	38	38	0	0	1	1	1	0.07585818	0	0.05	22	20	20	0	0.4	0	1	0	1								
2	39	39	0	0	0.6	1	1	0.04742587	0	0.05	22	20	20	0	0.4	0	0.6	0	1								
2	40	40	0	0	0.3	1	1	0.02931223	0	0.05	22	20	20	0	0.4	0	0.3	0	1								
2	41	41	0	0	0.1	1	1	0.01798621	0	0.05	22	20	20	0	0.4	0	0.1	0	1								
2	42	42	0	0																							

The tests for mutations: dynamic example



$$\begin{aligned}m &= 0.5 \\u_0 &= 0.5 \\u_s &= 0.5\end{aligned}$$

The tests for mutations: dynamic example



$$\begin{aligned}m &= 0.5 \\u_0 &= 0.5 \\u_s &= 0.5\end{aligned}$$

The tests for mutations: changed the code

MUTATION						
<i>Ga</i>	<i>Destroyed Genes / ALL Genes</i>	<i>Gi</i>	<i>Gim</i>	-	<i>Gd</i>	<i>Gb</i>
$H_a = \sum_k (G_a)_k$	<i>x-mutation density</i>	$H_i = \sum_k (G_i)_k$	$H_{im} = \sum_k (G_{im})_k$	-	$H_d = \sum_k (G_d)_k$	$H_b = \sum_k (G_b)_k$
<i>Apoptosis</i> $a' = a - H_a$ $a = \frac{1}{1 + e^{-s_0(x-0.5)}}$	<i>Hayflick limit (immortalization)</i> $i' = 1 - H_i$	<i>Invasion/metastasis transformation</i> $im' = H_{im}$	<i>Environmental death</i> k'		<i>Division process</i> $d' = \begin{cases} d - E' \cdot N & , \text{for normal cells} \\ d & , \text{for metastasis cells} \end{cases}$ $E' = \frac{E_0}{1 + F_0 \cdot H_b}$ and $d = H_d$ N – number of normal cells	

Mutation of driver genes (and Hallmarks variable and mutation rate) depends on probabilities u_S and u_O (see the table) for oncogenes and suppressors.

Mutation of passenger genes depends on probabilities $(1-u_S)$ and $(1-u_O)$ without changes in Hallmarks variables for oncogenes and suppressors.

Mutation occurs only during division process, so the mutation must occurs ONLY for parents and children independently. ONLY FOR TEST we changed the code and switch off the death of cell in order to check the mutation process.

The tests for mutations: gene files examples

GA	1000	apoptosis	o	1
GB	1	angiogenesis	o	1
GIM	1	invasion	o	1
GI	1	immortalization	o	1
GD	1	growth	o	1

GA	1	apoptosis	s	1
GB	1000	angiogenesis	s	1
GIM	1	invasion	s	1
GI	1	immortalization	s	1
GD	1	growth	s	1

The tests for mutations: cell init file

The celling file is constant, it has the cells with all combinations of 4 genes + GD. The GD gene is needed, because the mutation occurs ONLY during the division process, what is why we need GD (GD switch on the division process with the probability 1).

Cellin file:

```
1  GA
2  GI
3  GD
4  GB
5  GIM
6
7  GD,GA
8  GD,GA,GI
9  GD,GA,GI,GB
10  GD,GA,GI,GB,GIM
11  GD,GA,GB
12  GD,GA,GB,GIM
13  GD,GA,GIM
14  GD,GI
15  GD,GI,GB
16  GD,GI,GB,GIM
17  GD,GB
18  GD,GB,GIM
19  GD,GIM
20  GD,GA,GI,GIM
21  GD,GI,GIM
22  GD,GA
23  GD,GI
24  GD,GB
25  GD,GIM
```

The tests for mutations: output data

		Genefile		U_s	U_o	m_0	Results
GA	1	apoptosis	o	1			<p>The cell divisions with the HD Hallmarks without mutations.</p> <p>Please, check the mutation rate.</p> <p>Conclusion: for oncogenes without mutation.</p>
GB	1	angiogenesis	o	1			<p>Same as provisos.</p> <p>Conclusion: for suppressor same</p>
GIM	1	invasion	o	1			
GI	1	immortalization	o	1			
GD	1	growth	o	1			
GA	100	apoptosis	o	1			<p>The mutation occurs only in the passenger part of genes.</p>
GB	100	angiogenesis	o	1			
GIM	100	invasion	o	1			
GI	100	immortalization	o	1			
GD	100	growth	o	1			

The tests for mutations: output data

Genefile					U_s	U_o	m_o	Results
GA	100	apoptosis	s	1				
GB	100	angiogenesis	s	1				
GIM	100	invasion	s	1	0	0	1	The mutation occurs only in the passenger part of genes.
GI	100	immortalization	s	1				
GD	100	growth	s	1				
GA	100	apoptosis	o	1				
GB	100	angiogenesis	o	1				
GIM	100	invasion	o	1	0	1	1	The mutation occurs only in the driver part of genes.
GI	100	immortalization	o	1				
GD	100	growth	o	1				
GA	100	apoptosis	s	1				
GB	100	angiogenesis	s	1				
GIM	100	invasion	s	1	0	1	1	The mutation occurs only in the passenger part of genes, because of $U_s=0$.
GI	100	immortalization	s	1				
GD	100	growth	s	1				
GA	100	apoptosis	o	1				
GB	100	angiogenesis	o	1				
GIM	100	invasion	o	1	1	0	1	The mutation occurs only in the passenger part of genes, because of $U_o=0$.
GI	100	immortalization	o	1				
GD	100	growth	o	1				

The tests for mutations: output data

Genefile					U_s	U_o	m_o	Results
GA	100	apoptosis	s	1				
GB	100	angiogenesis	s	1				
GIM	100	invasion	s	1				
GI	100	immortalization	s	1				
GD	100	growth	s	1				
					1	0	1	The mutation occurs only in the driver part of genes.
GA	100	apoptosis	o	1				
GB	100	angiogenesis	o	1				
GIM	100	invasion	o	1				
GI	100	immortalization	o	1				
GD	100	growth	o	1				
					1	1	1	The mutation occurs only in the driver part of genes.
GA	100	apoptosis	s	1				
GB	100	angiogenesis	s	1				
GIM	100	invasion	s	1				
GI	100	immortalization	s	1				
GD	100	growth	s	1				
					1	1	1	The mutation occurs only in the driver part of genes.
GA	100	apoptosis	o	1				
GB	100	angiogenesis	o	1				
GIM	100	invasion	o	1				
GI	100	immortalization	o	1				
GD	100	growth	o	1				
					0.5	0.5	1	The mutation occurs in the driver and passenger parts of genes.

The tests for mutations: output data

Genefile					U_s	U_o	m_0	Results
GA	100	apoptosis	s	1				The mutation occurs in the driver and passenger parts of genes.
GB	100	angiogenesis	s	1				
GIM	100	invasion	s	1	0.5	0.5	1	
GI	100	immortalization	s	1				
GD	100	growth	s	1				
GA	1000	apoptosis	o	1				The mutation occurs only in the driver and passenger parts of gene with a <u>longest</u> CDS.
GB	1	angiogenesis	o	1				
GIM	1	invasion	o	1	0.5	0.5	0.001	
GI	1	immortalization	o	1				
GD	1	growth	o	1				
GA	1	apoptosis	s	1				The mutation occurs only in the driver and passenger parts of gene with a <u>longest</u> CDS.
GB	1000	angiogenesis	s	1				
GIM	1	invasion	s	1	0.5	0.5	0.001	
GI	1	immortalization	s	1				
GD	1	growth	s	1				