

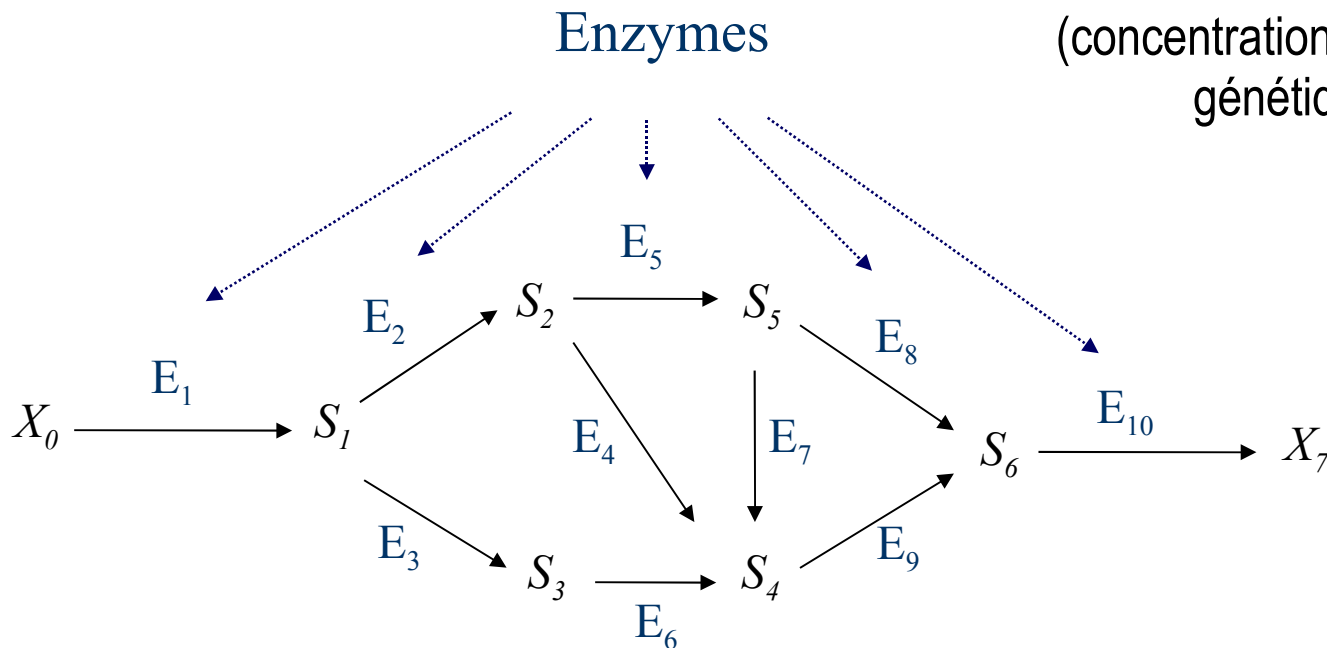
Evolution des concentrations d'enzymes dans les systèmes métaboliques

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Modélisation de la relation génotype-phénotype : la théorie du contrôle métabolique

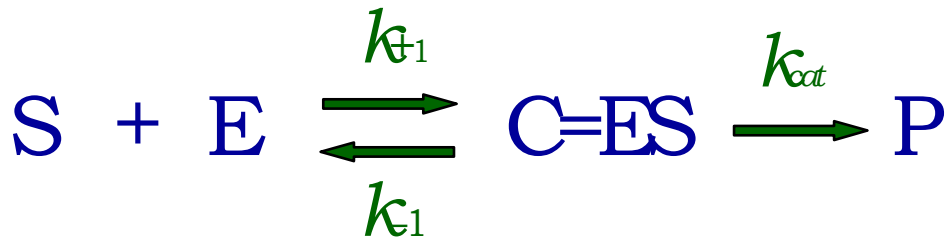
Le “génotype” : tous les gènes qui contrôlent l'activité des enzymes (concentrations, paramètres cinétiques, génétiquement variables)



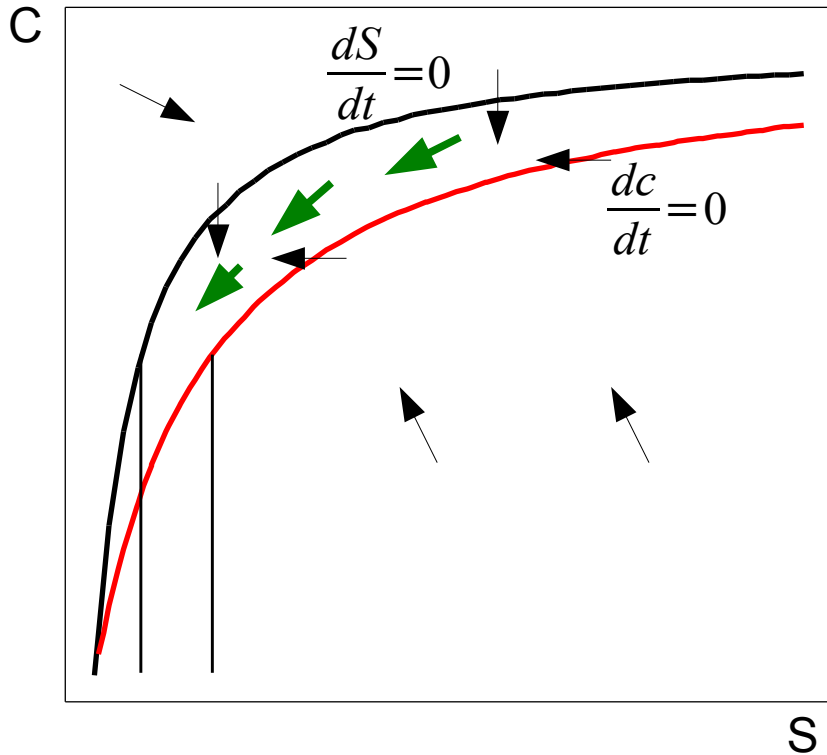
Le “phénotype” : le flux J

- 1903 : Victor Henri : la formation du complexe enzyme-substrat est l'étape essentielle du mécanisme catalytique
- 1913 : Michaelis & Menten proposent les équations de vitesse
- 1973-1974 : Kacser & Burns et Heinrich et Rapoport proposent la théorie du contrôle métabolique

Cinétique enzymatique



$$\left\{ \begin{array}{l} \frac{ds}{dt} = -k_1 es + k_{-1} c \\ \frac{de}{dt} = -k_1 es + k_{-1} c + k_{cat} c \\ \frac{dc}{dt} = k_1 es - k_{-1} c - k_{cat} c \\ \frac{dp}{dt} = k_{cat} c \end{array} \right.$$



Étape rapide

Étape lente = état quasi -stationnaire

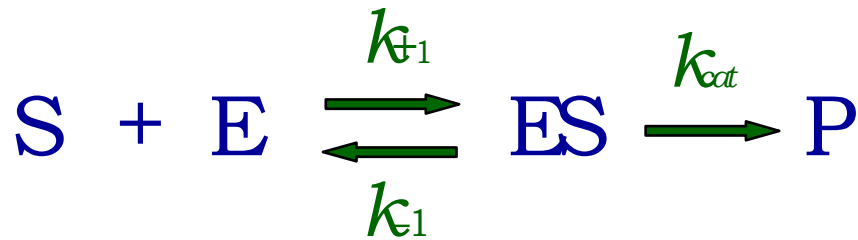
$$v \approx \frac{k_{cat} E S}{K_m + S}$$

$$K_m = \frac{k_{-1} + k_{cat}}{k_1}$$

Il existe, au sein d'une cellule, des temps longs et des temps courts.

Après une phase rapide d'ajustement, les concentrations de C et S ne changent « plus »

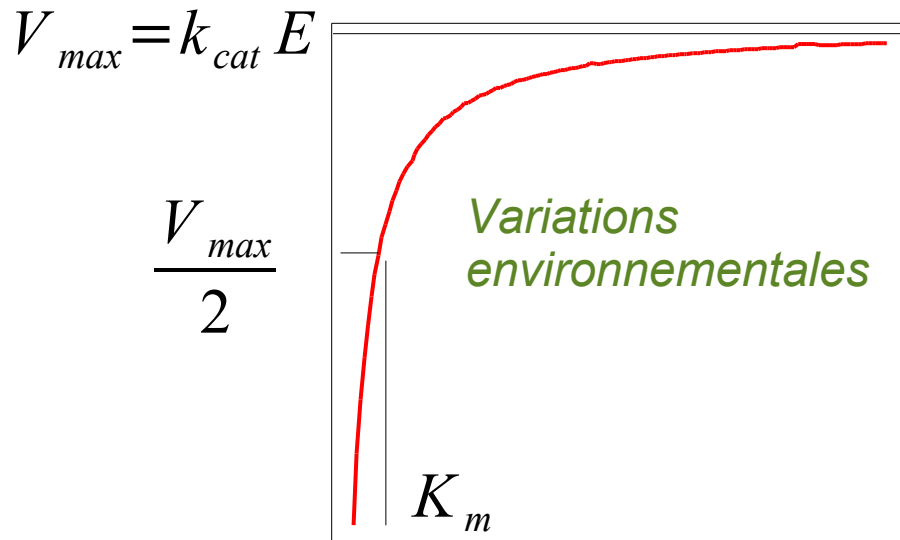
Cinétique enzymatique



$$v = \frac{k_{cat} E S}{K_m + S}$$

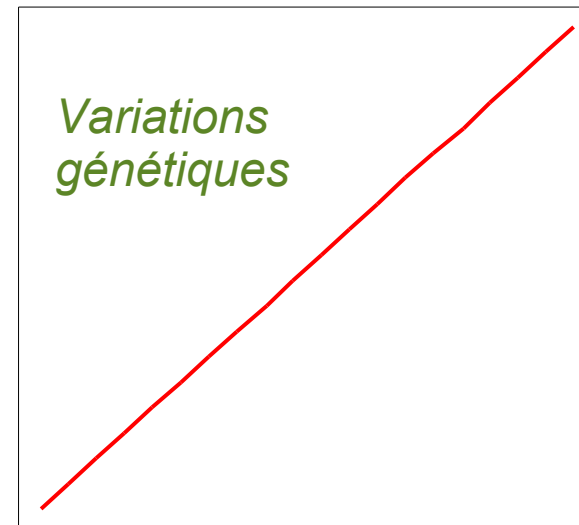
$$K_m = \frac{k_{-1} + k_{cat}}{k_{+1}}$$

Vitesse de la réaction



Concentration de S

Vitesse de la réaction



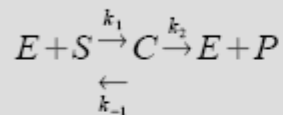
Concentration de E

La vitesse d'une réaction enzymatique n'est pas affectée de la même manière par des variations de concentrations en métabolites et des variations de concentrations d'enzymes.

La cellule n'est pas un environnement homogène. Obstacles à la diffusion des molécules ?

Michaelis-Menten enzyme kinetics

- Mass-action laws (biochemical textbooks)

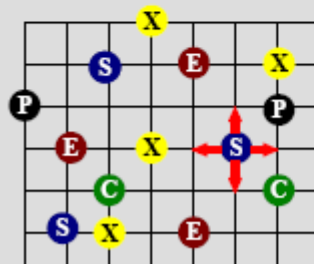


$$\frac{d\rho_C}{dt} = k_1\rho_E\rho_S - (k_{-1} + k_2)\rho_C$$

$$\frac{d\rho_S}{dt} = -k_1\rho_E\rho_S + k_{-1}\rho_C$$

$$\frac{d\rho_P}{dt} = k_2\rho_C$$

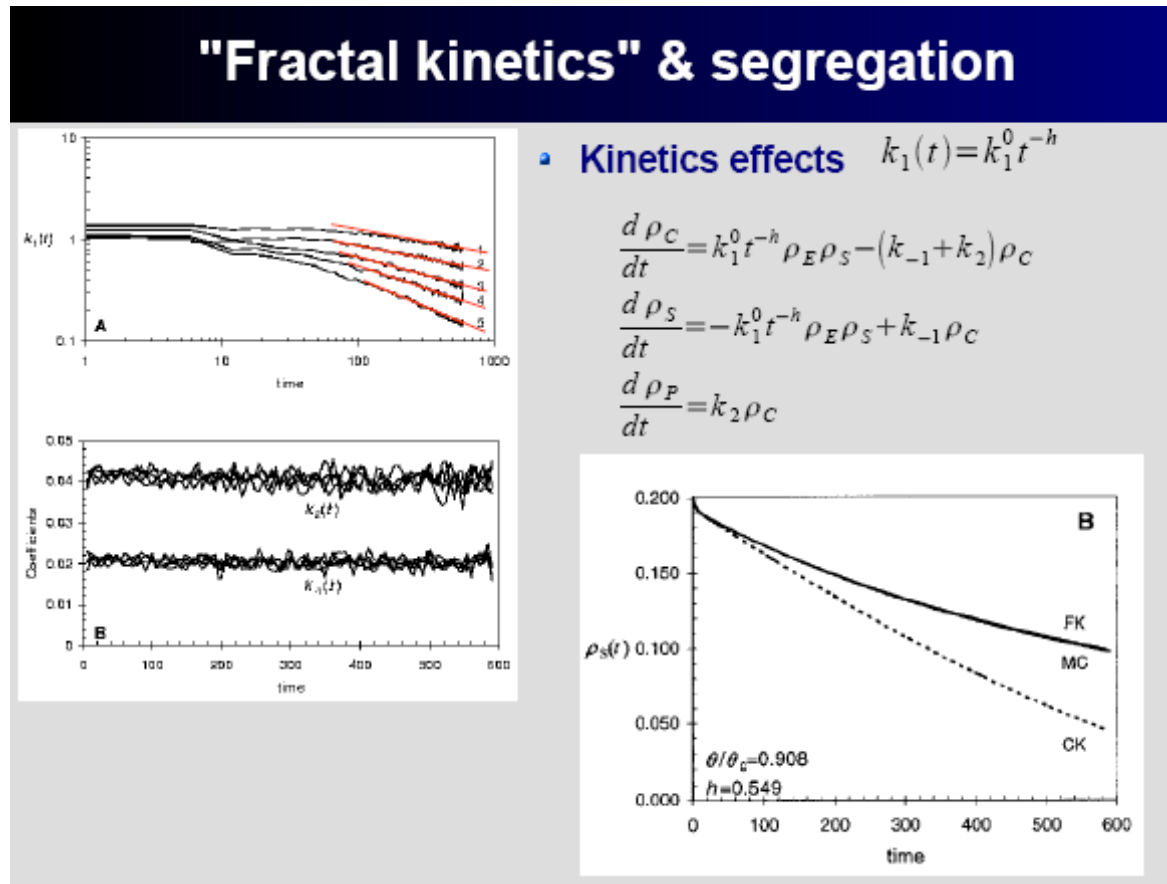
- "Multi-agent" simulations (reactive lattice gas)



- E-S encounters yield C with proba $\alpha \sim k_1$
- C yields E & S with proba $\beta \sim k_{-1}$
- C yields E & P with proba $\gamma \sim k_2$

(Hugues Berry, INRIA Futurs)

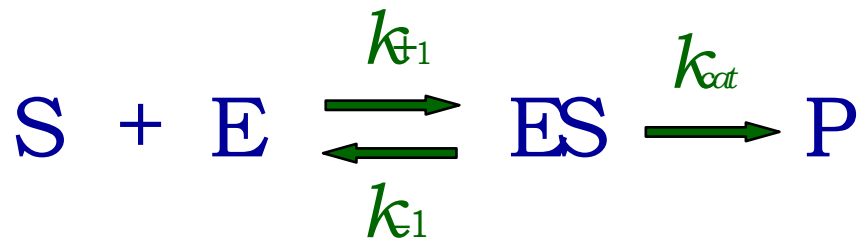
La constante de vitesse k_1 diminue au cours du temps



(Hugues Berry, INRIA Futurs)

Cinétique enzymatique

Une réaction :



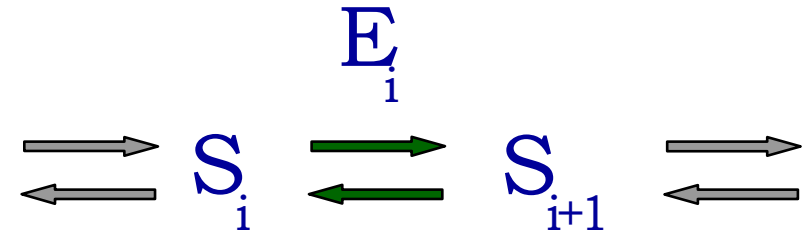
$$v = \frac{k_{cat} E S}{K_m + S}$$

$$K_m = \frac{k_{-1} + k_{cat}}{k_{+1}}$$

Hypothèses :
 état stationnaire +
 les enzymes fonctionnent loin
 de la saturation
 ($S_i \ll K_{m_i}$)

Hypothèse :
 état stationnaire : $v_1 = v_2 = \dots = v_n$

Une chaîne de réactions :

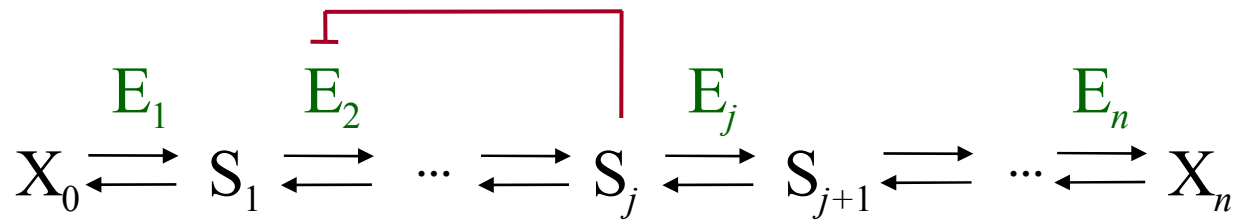


$$v_i = \frac{k_{cat} E_i (S_i - \frac{S_{i+1}}{K_{i,i+1}})}{1 + \frac{S_i}{K_{m_i}} + \frac{S_{i+1}}{K_{m_{i+1}}}}$$



$$v_i \approx \frac{k_{cat} E_i (S_i - \frac{S_{i+1}}{K_{i,i+1}})}{K_{m_i}}$$

Théorie du contrôle métabolique



Concentrations externes

$$J = \frac{X_0 - \frac{X_n}{K_{0,n}}}{\sum_{j=1}^n \frac{1}{K_{0,j+1} \frac{k_{cat_j}}{K_{m,j}} E_j}}$$

Concentration enzyme j

Paramètres cinétiques

$$J = \frac{X}{\sum_{j=1}^n \frac{1}{A_j E_j}}$$

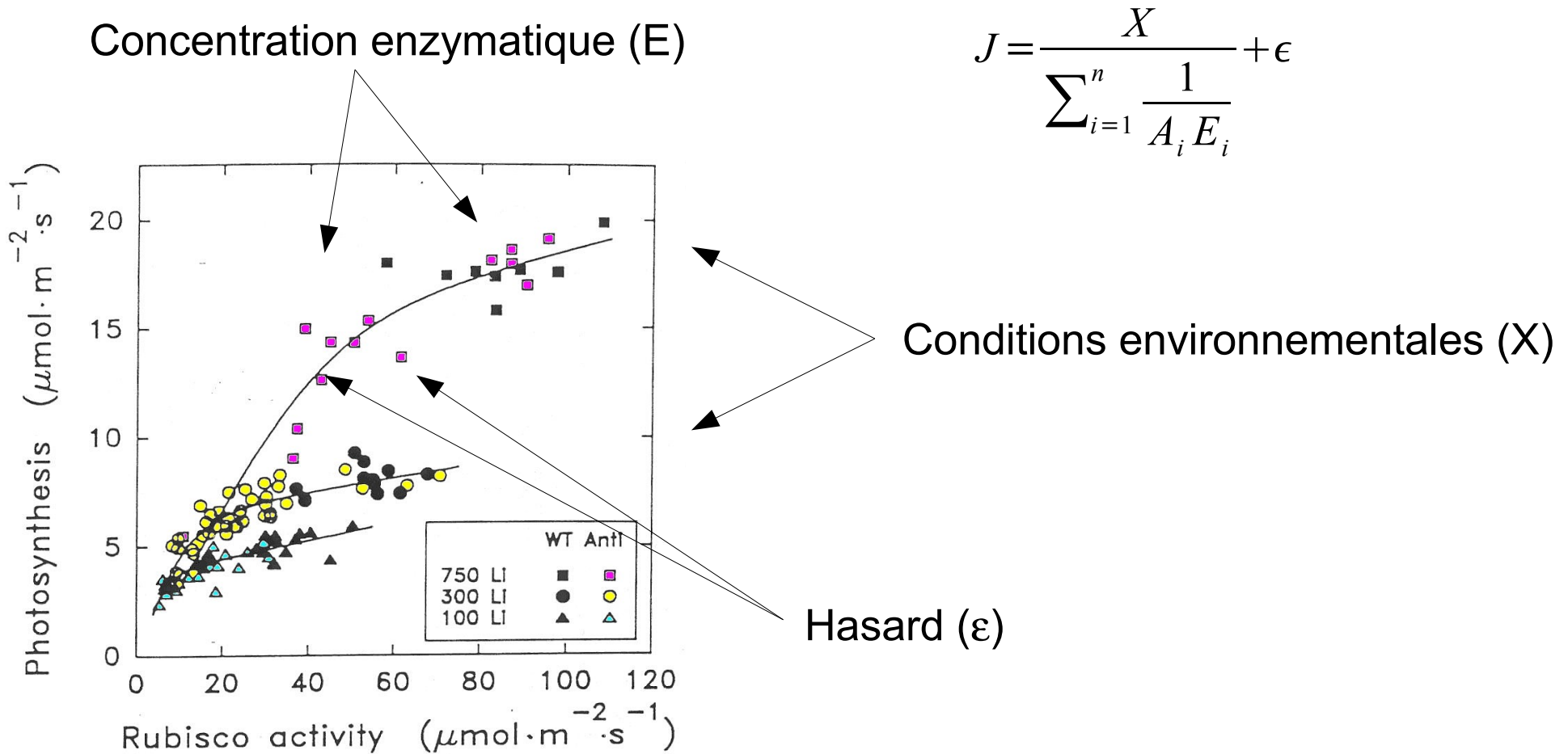
Kacser H. & J. A. Burns 1973. *Symp. Soc. Exp. Biol.* 27: 65.

Heinrich R. & T.A. Rapoport 1974. *Eur. J. Biochem.* 42: 89.

Sen AK. (1996) *J Theor Biol.* 182:269-75.

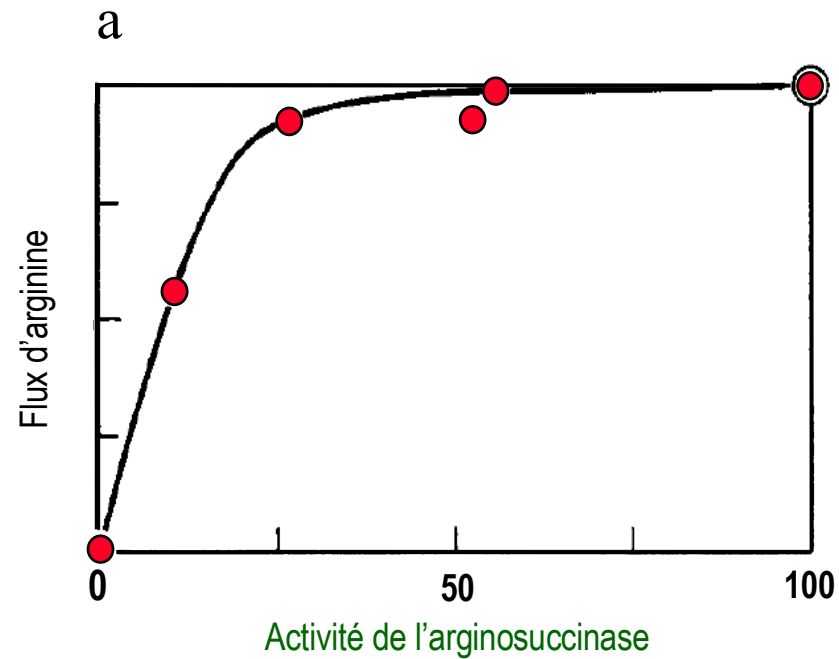
Le hasard environnemental et physiologique

Réponse du flux (le phénotype) aux variations des paramètres enzymatiques (le génotype) : exemple



Réponse du flux (le génotype) aux variations des paramètres enzymatiques
(le génotype) : Une seule enzyme variable :

$$J = \frac{X}{b + \frac{1}{A_i E_i}}$$



Le hasard génétique : mutations

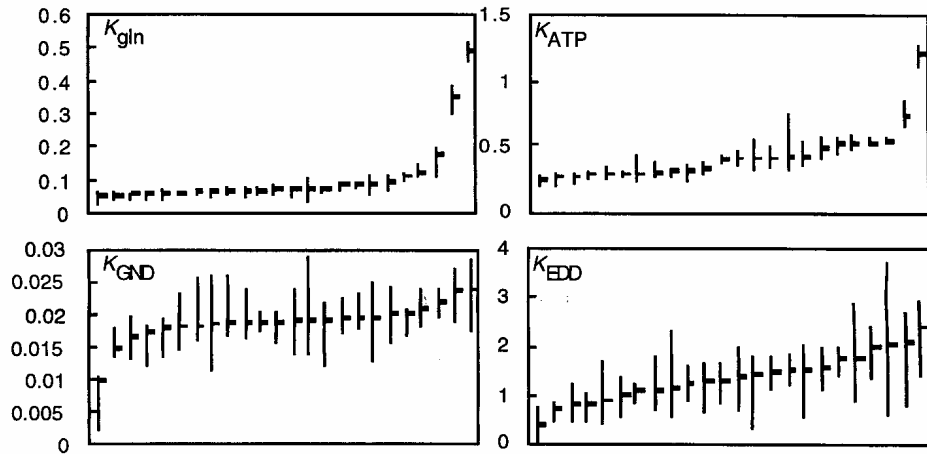
$$J = \frac{X}{\sum_{i=1}^n \frac{1}{A_i E_i}} + \epsilon$$

Propriétés cinétiques

concentrations

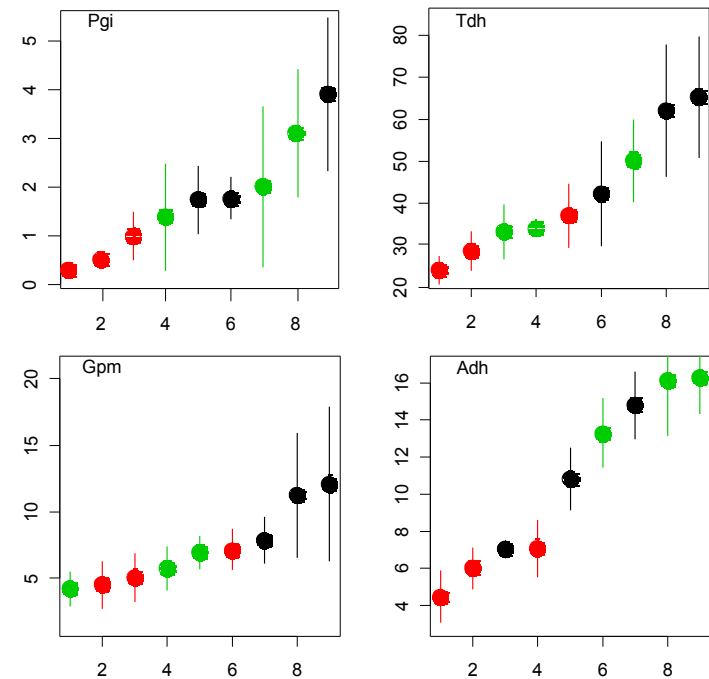
Les propriétés cinétiques des enzymes et leurs concentrations n'évoluent pas forcément à la même vitesse

Variation génétique des propriétés cinétiques des enzymes chez E. Coli



Wang & Dykhuisen, 2001. Pathway of gluconate metabolism in E. coli. Evolution, 55:897.

Variation génétique des quantité d'enzymes de la glycolyse chez la levure



Grima et al, in prep

Les concentrations des enzymes sont plus variables que leurs paramètres cinétiques

Change of a mutant allele frequency due to selection (haploid population)

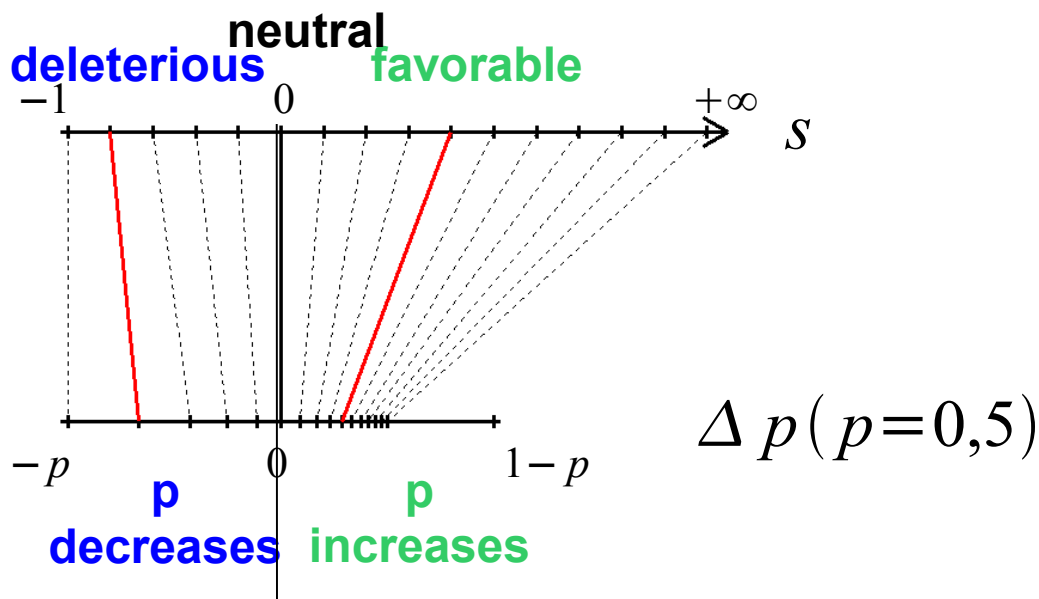
	Genotype	
	WT	M
fitness (nb offsprings)	ω^{WT}	ω^M
fréquency	$1-p$	p

Selection coefficient :

$$s = \frac{(\omega^M - \omega^{WT})}{\omega^{WT}}$$

Change in the frequency of allele M :

$$\Delta p = p(1-p) \frac{s}{(1+ps)}$$



Asymmetrical effects of selection on allele frequencies

Mutations in a metabolic system

$$J^{WT} = \frac{X E_i^{WT}}{b_i + E_i^{WT}}$$

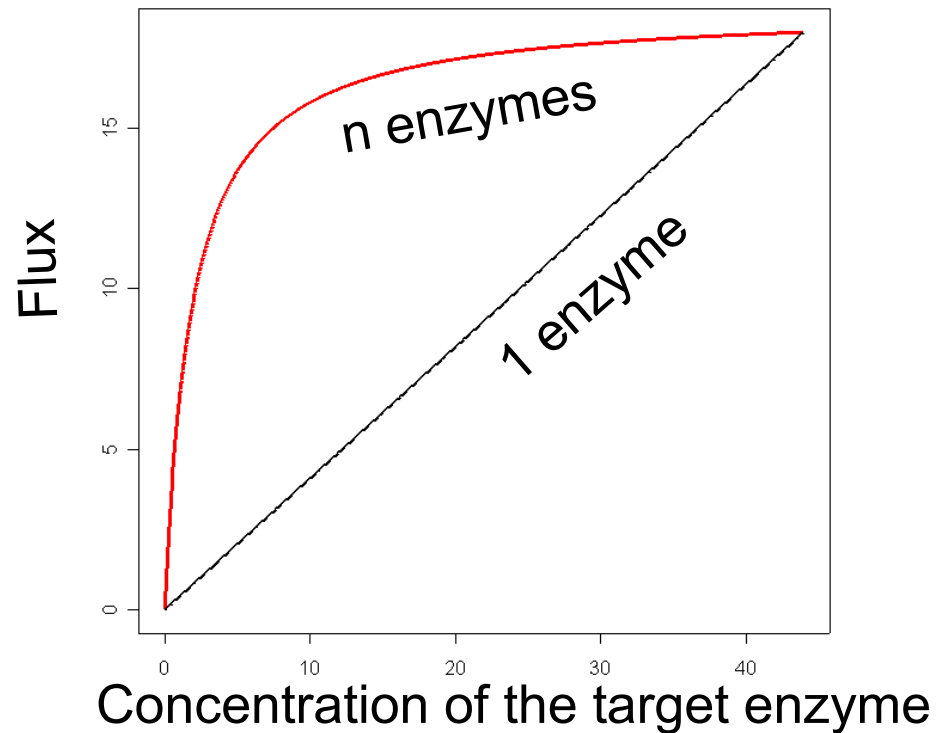
Other enzymes = genetic background
 Target enzyme

$$b_i = \frac{1}{A_i} / \left(\sum_{j \neq i} \frac{1}{A_j E_j} \right)$$

$$J^M = \frac{X (E_i^{WT} + (\Delta E)_i)}{b + E_i^{WT} + (\Delta E)_i}$$

$$s = \frac{(J^M - J^{WT})}{J^{WT}}$$

Hyp = fitness is proportional to flux

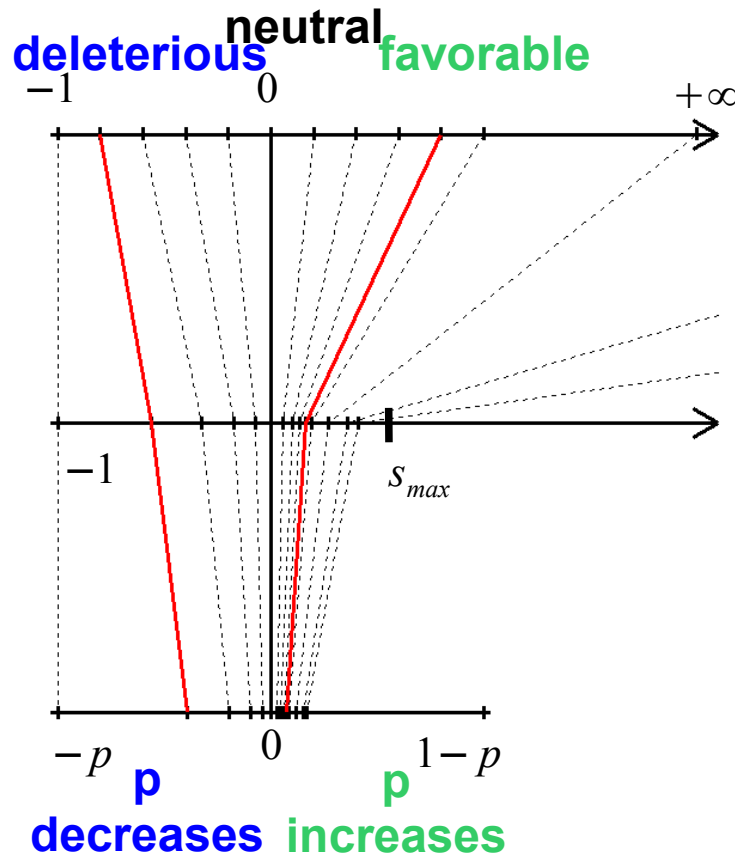


Evolution of a mutant allele frequency in a metabolic system

$$\left(\frac{\Delta E_i}{E_i}\right) = \frac{(E_i^M - E_i^{WT})}{E_i^{WT}}$$

$$s_i = \left(\frac{\Delta J}{J}\right)_i = \frac{b_i \Delta E_i}{E_i^{WT} [b_i + E_i^{WT} + \Delta E_i]}$$

$$\Delta p = p(1-p) \frac{s}{(1+ps)}$$



Knowing the effect of a mutation on enzyme concentration

We calculate the selection coefficient of the mutant allele

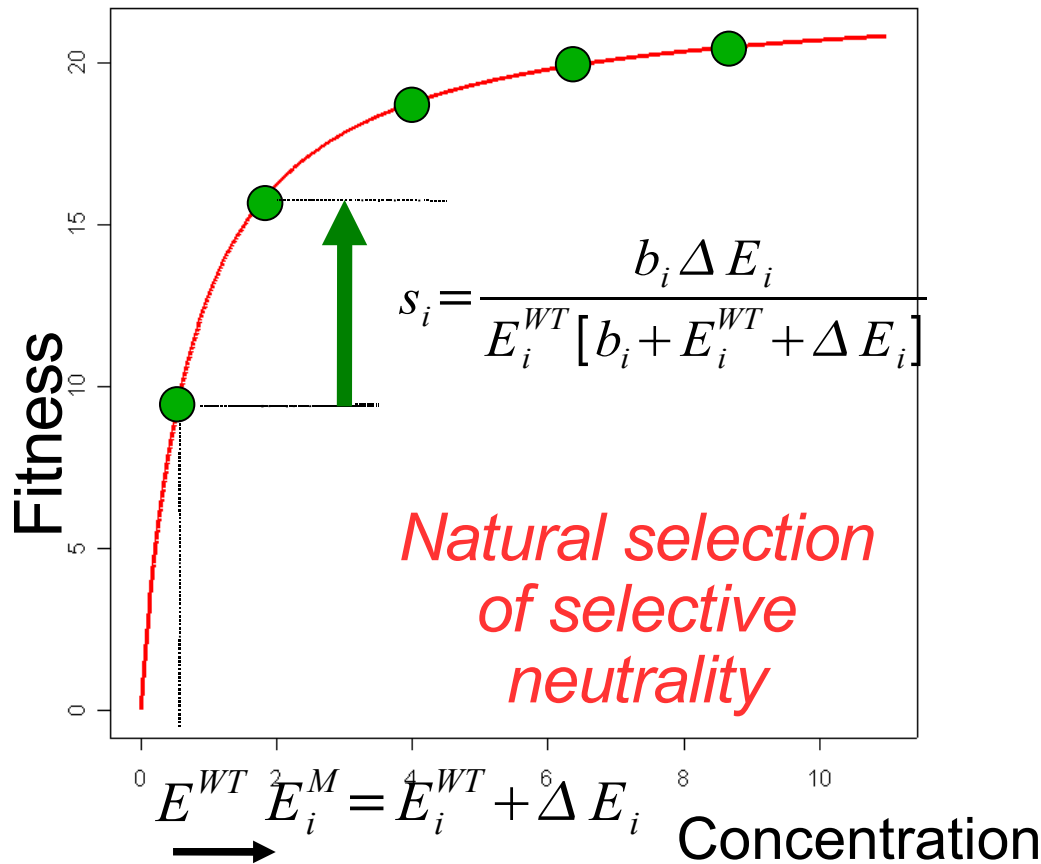
And the corresponding variation of the mutant allele frequency

The asymmetry of mutation effects on fitness increases the asymmetry of the effect of selection on allele frequency

Short term evolution : One variable locus

Effect of mutation on enzyme concentration:

$$E_i^M = E_i^{WT} \pm \Delta E_i$$

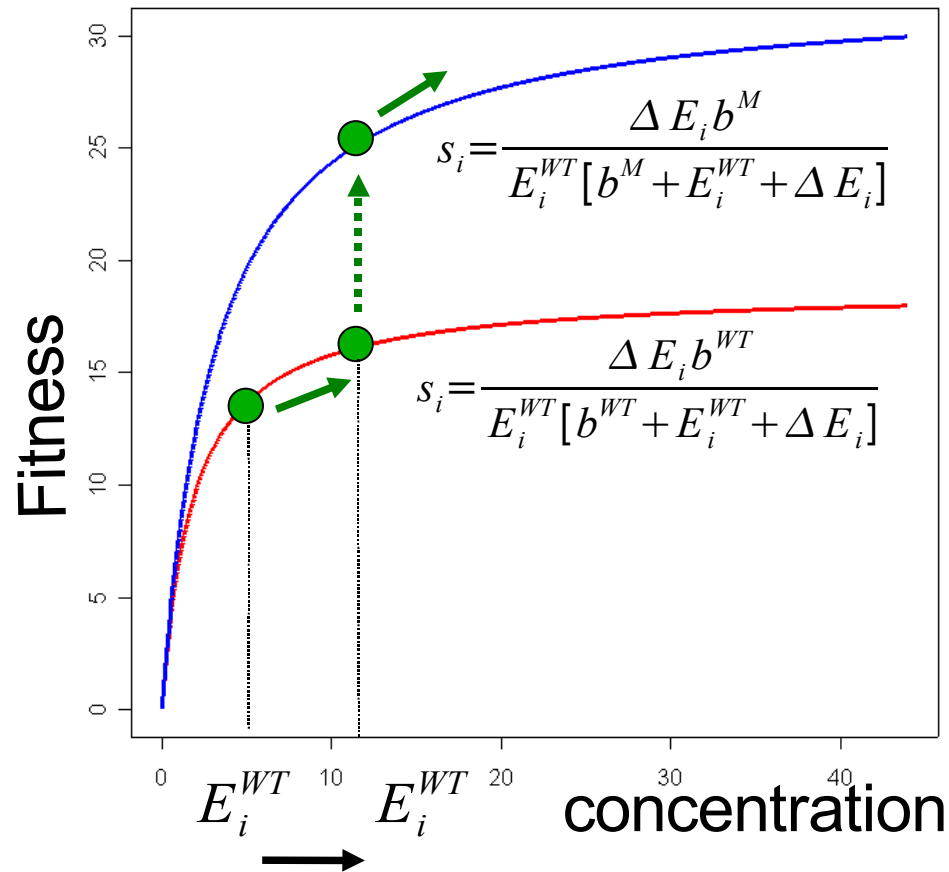


The selection coefficient of a favourable mutation decreases as the enzyme concentration increases

(Hartl, Dykhuizen and Dean, 1985)

Short term evolution: multilocus metabolic system

Targets of selection are multiple ...



The fitness effect of a mutation on one enzyme depends on the genetic background = on the concentration of the other enzymes of the system

Le hasard génétique : mutations et sélection

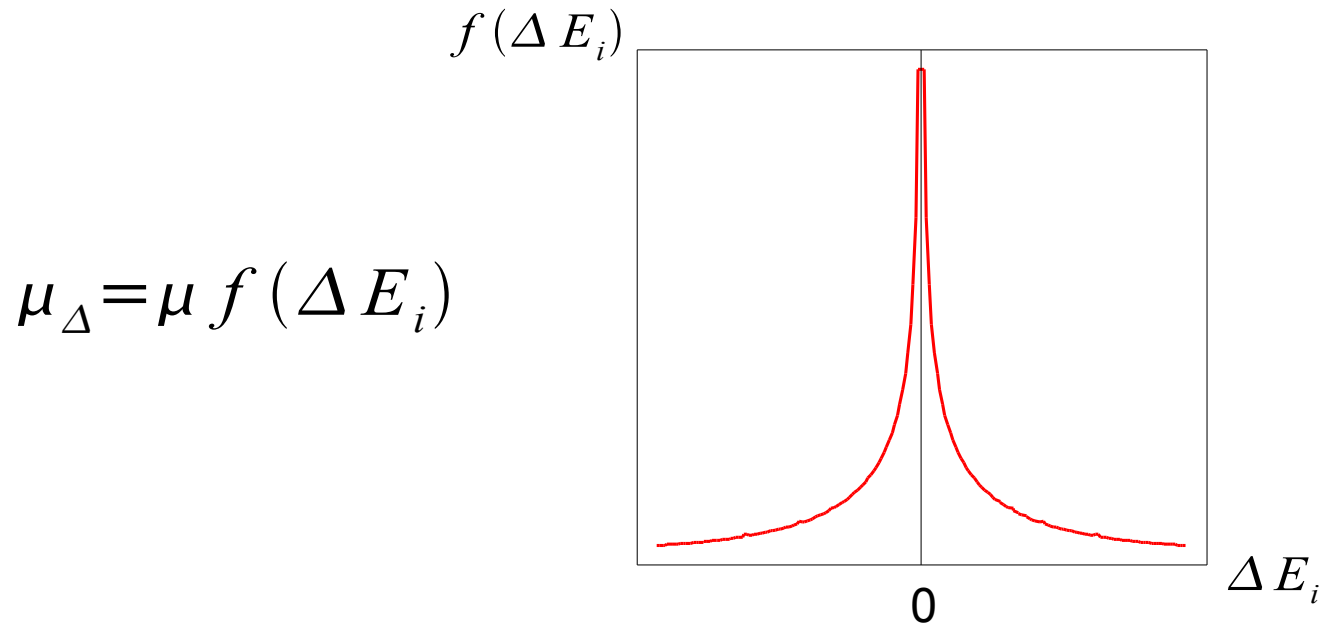
Devenir d'une mutation affectant la concentration d'une enzyme dans une population

	Génotype WT	M
concentration enzyme	E_i^{WT}	$E_i^M = E_i^{WT} + \Delta E_i$
fréquence	$p_0 = (1 - \mu_\Delta) p$	$q_0 = \mu_\Delta p$
flux	J^{WT}	J^M
valeur sélective (nb descendants)	w^{WT}	w^M

$$\Delta E_i$$

est une variable
aléatoire à laquelle on
peut associer une loi
de probabilité

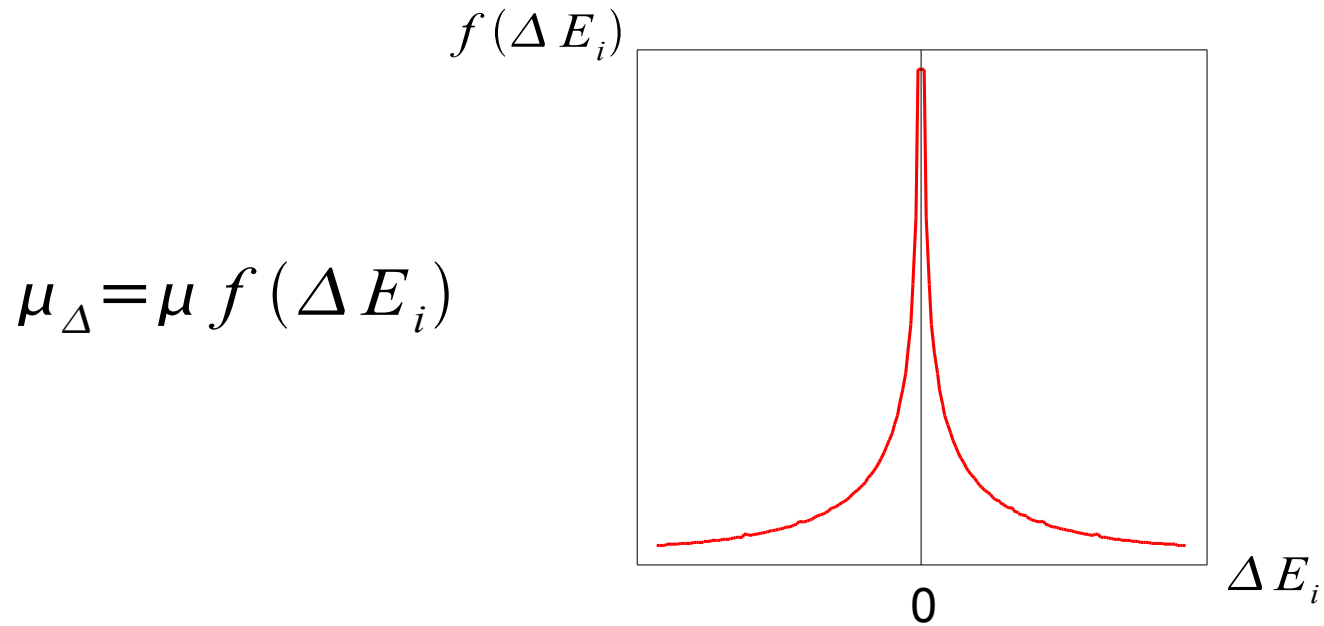
Distribution des effets d'une mutation



Une mutation se produit sur l'enzyme i avec une probabilité μ et change sa concentration d'un facteur ΔE avec une probabilité $f(\Delta E)$

On ne peut pas dissocier le taux de mutation de son effet sur l'enzyme

Distribution des effets d'une mutation



Modèle additif : $|\Delta E_i| \approx \Gamma(a, b)$ $\langle |\Delta E_i| \rangle = m$

Modèle à effets constants : $\frac{|\Delta E_i|}{E_i} \approx \Gamma(a, b)$ $\langle |\Delta E_i| \rangle = m E_i$

Long term evolution: multilocus metabolic system

Hypothesis : Evolution through stepwise fixation of mutations (probability μ) of weak effect (δ) that change one enzyme concentration at a time

Fixation probability of a mutation : $P_{fix} = \frac{2s_i}{1 - e^{-2Ns_i}} \simeq 2s_i$

Change in enzyme concentration during time interval dt : $E_i(t + \delta t) = 2\mu s_i E_i^M + (1 - 2\mu s_i) E_i^{WT}$

$$\frac{\partial E_i}{\partial t} \approx 2\mu s_i (\delta E)_i$$

Conditions for an equilibrium state : $\{\forall i, \frac{\partial E_i}{\partial t} = 0\}$

Sélection pour augmenter le flux : recherche d'un état d'équilibre

$$J = \frac{X E_{TOT}}{\sum_{i=1}^n \frac{1}{A_i e_i}} + \epsilon \quad \forall i, \quad \frac{\partial E_i}{\partial t} = 0 \Leftrightarrow \begin{cases} \frac{\partial E_{TOT}}{\partial t} = 0 \\ \forall i, \quad \frac{\partial e_i}{\partial t} = 0 \end{cases}$$

Evolution des concentrations relatives :

$x = \ln(E_{TOT})$ augmente au cours du temps

$$\forall i, \quad \frac{\partial e_i}{\partial t} = 0 \Leftrightarrow \forall i, \quad \frac{\partial e_i}{\partial x} = 0 \Leftrightarrow \forall i, \quad \frac{\frac{\partial E_i}{\partial t}}{\frac{\partial E_{TOT}}{\partial t}} - e_i = 0$$

Sélection pour augmenter le flux : recherche d'un état d'équilibre

$$\frac{\partial E_i}{\partial t} = 2\mu E_{TOT} s_i (\delta e)_i$$

$$\frac{\partial E_{TOT}}{\partial t} = 2\mu E_{TOT} \sum_j s_j (\delta e)_j$$


Pour des mutations de faible
effet

Pour des enzymes indépendantes

*Effet d'un changement de E_i
sur E_k*



$$s_i = \frac{\Delta J}{J} \approx \frac{1}{J} \sum_k \frac{\partial J}{\partial E_k} (\delta E)_{ik}$$

$$s_i \approx \left(\frac{E_i}{J} \frac{\partial J}{\partial E_i} \right) \left(\frac{(\delta e)_i}{e_i} \right)$$


Coefficient de contrôle de E_i

$$s_i \approx \left(\frac{\frac{1}{A_i e_i}}{\sum_j \frac{1}{A_j e_j}} \right) \left(\frac{(\delta e)_i}{e_i} \right)$$

Sélection pour augmenter le flux : recherche d'un état d'équilibre

$$\forall i, \frac{\frac{1}{A_i} \frac{(\delta e)_i^2}{e_i^2}}{\sum_j \frac{1}{A_j} \frac{(\delta e)_j^2}{e_j^2}} = e_i$$

Modèle additif :

$$|\Delta E_i| \approx \Gamma(a, b)$$

$$\langle |\Delta E_i| \rangle = m$$

$$e_i^{eq} = \frac{\frac{1}{A_i^{(-1/3)}}}{\sum_j \frac{1}{A_j^{(-1/3)}}$$

Quel que soit le modèle de mutation, il existe un équilibre stable, dans lequel les proportions des enzymes sont déterminées par leurs paramètres cinétiques

Modèle invariant :

$$\frac{|\Delta E_i|}{E_i} \approx \Gamma(a, b)$$

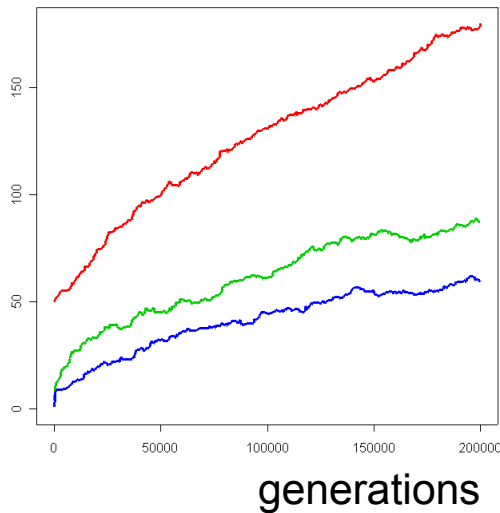
$$\langle |\Delta E_i| \rangle = m E_i$$

$$e_i^{eq} = \frac{\frac{1}{A_i^{(-1)}}}{\sum_j \frac{1}{A_j^{(-1)}}$$

Long term evolution : multilocus metabolic system

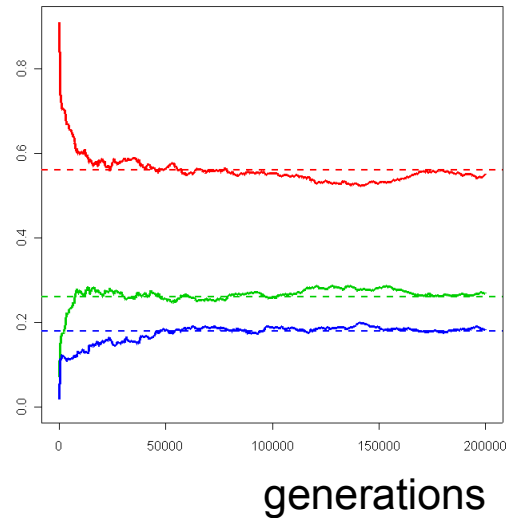
Additive model $E_i^M = E_i^{WT} \pm \delta$

Enzyme concentration



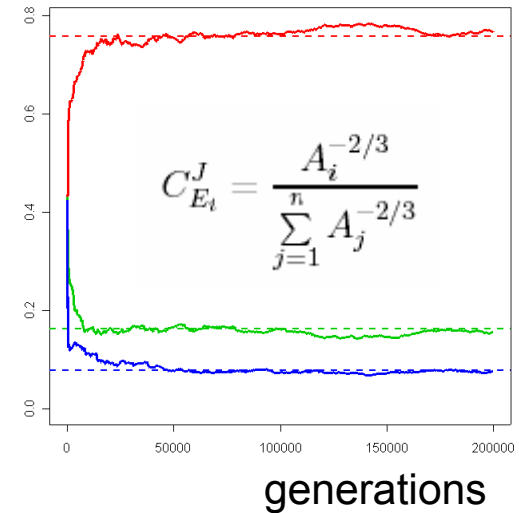
Selection to increase the flux
=> all enzyme concentrations increase...

Proportion of each enzyme



... in constant proportions
=> enzymes with the lowest activity are the most abundant

Flux control coefficient



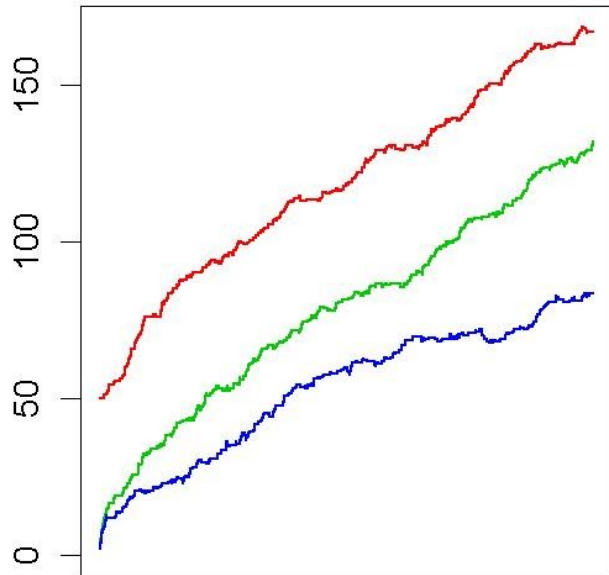
... each relative variation of their concentration has a more important effect on the flux

Natural selection shapes the evolution of enzyme concentrations in metabolic systems

- Natural selection determines the sharing out of the flux control in a linear metabolic system
- At evolutionary scales, predictions of the « additive » mutation model are an unequal sharing out of the control of the flux:
 - *The sharing out of the flux control is unstable over time: an enzyme may have an important effect, as long as a mutation does not change its kinetic parameters*

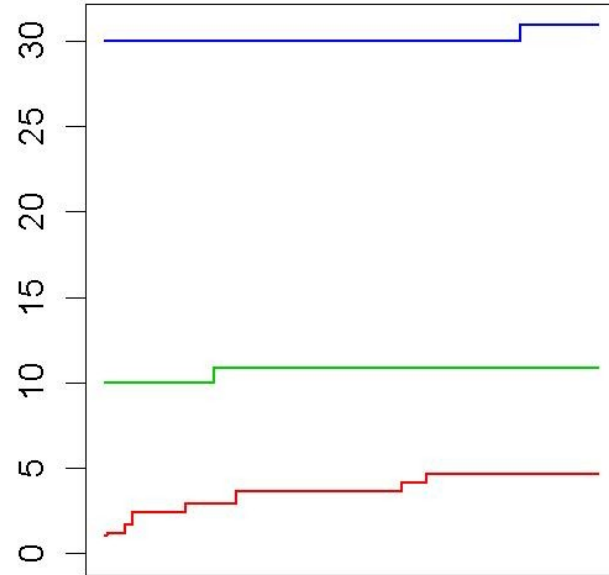
Evolution des activités des enzymes d'une voie métabolique sous l'effet d'une sélection pour augmenter le flux :

Concentration des enzymes



les concentrations de toutes les enzymes augmentent

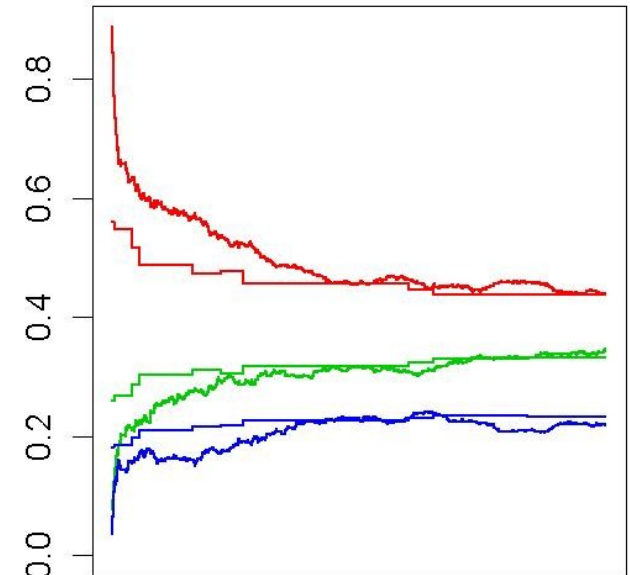
Paramètres cinétiques



temps

les paramètres cinétiques des enzymes ont un taux de mutation plus faible

Proportion de chaque enzyme



les concentrations des enzymes augmentent dans des proportions qui dépendent des paramètres cinétiques

Cas général : recherche d'un état d'équilibre

Une mutation sur l'enzyme cible i_0 change les concentrations de toutes les enzymes du système

$$E_j \longrightarrow E_j + (\delta E)_{i_0 j}$$

$$\frac{\partial E_j}{\partial t} \approx 2\mu \sum_{i_0} s_{i_0} (\delta E)_{i_0 j}$$

$$s_{i_0} = \frac{\Delta J}{J} \approx \frac{1}{J} \sum_k \frac{\partial J}{\partial E_k} (\delta E)_{i_0 k}$$

$$\frac{\partial E_j}{\partial t} \approx \frac{2\mu}{J} \sum_{i_0} \sum_k \frac{\partial J}{\partial E_k} (\delta E)_{i_0 j} (\delta E)_{i_0 k}$$

Taking into account constraints on
enzyme concentrations:
optimization

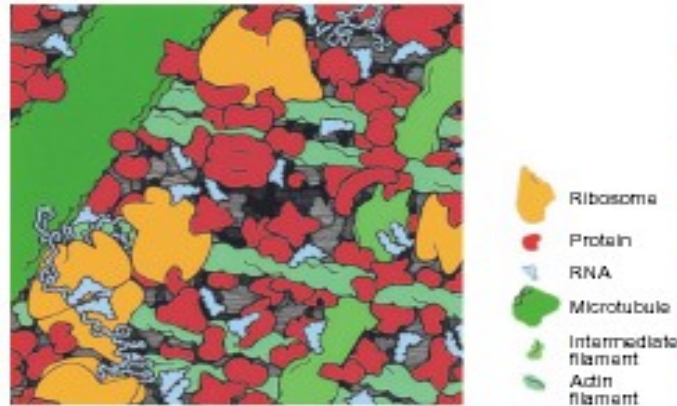
Taking into account constraints on enzyme concentration

I – Total enzyme content

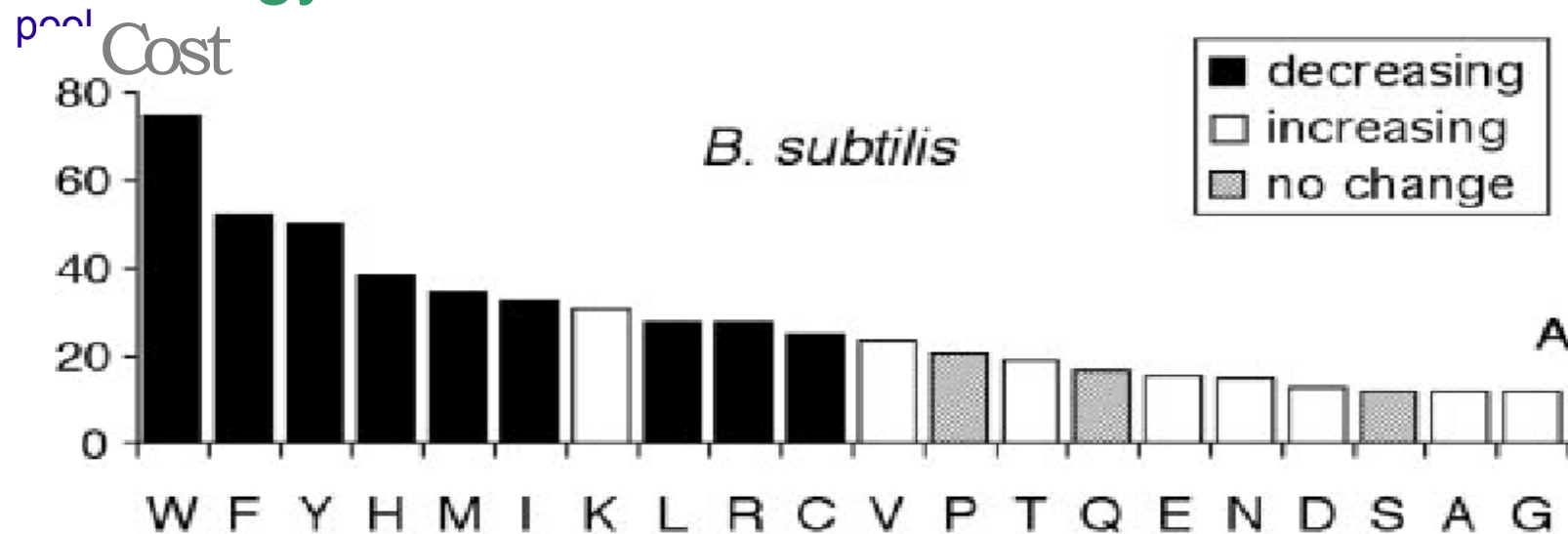
1- Space constraints

Limited cell/organelle volume \Rightarrow crowding

Ribosomes = 30-40% of cell volume;
 proteins = 20-30% of cell volume;
 solubility and diffusion limitation



2- Energy constraints (ATP/GTP) : maintaining the protein



Akašhi and Gojobori, 2002. Metabolic efficiency and amino acid composition in the proteomes of *Escherichia coli* and *Bacillus subtilis*. PNAS, 99:3695

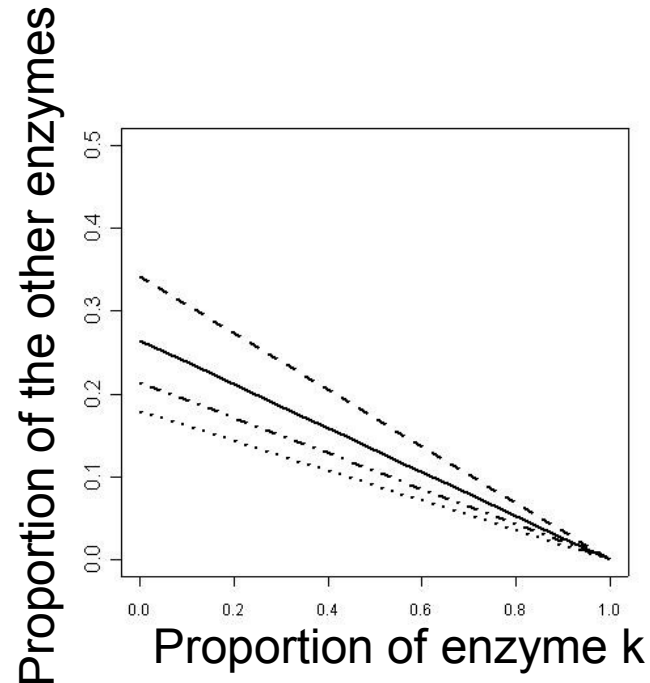
Taking into account constraints on enzyme concentration

I – Total enzyme content

The cell must limit enzyme content allocated to a given pathway

$$\sum_{i=1}^n Q_i = Q_{Tot} = \text{Constant}$$

Redistribution
coefficient (slope):
 $\alpha_{ij} = \delta Q_j / \delta Q_i = -Q_j / (Q_{Tot} - Q_i)$

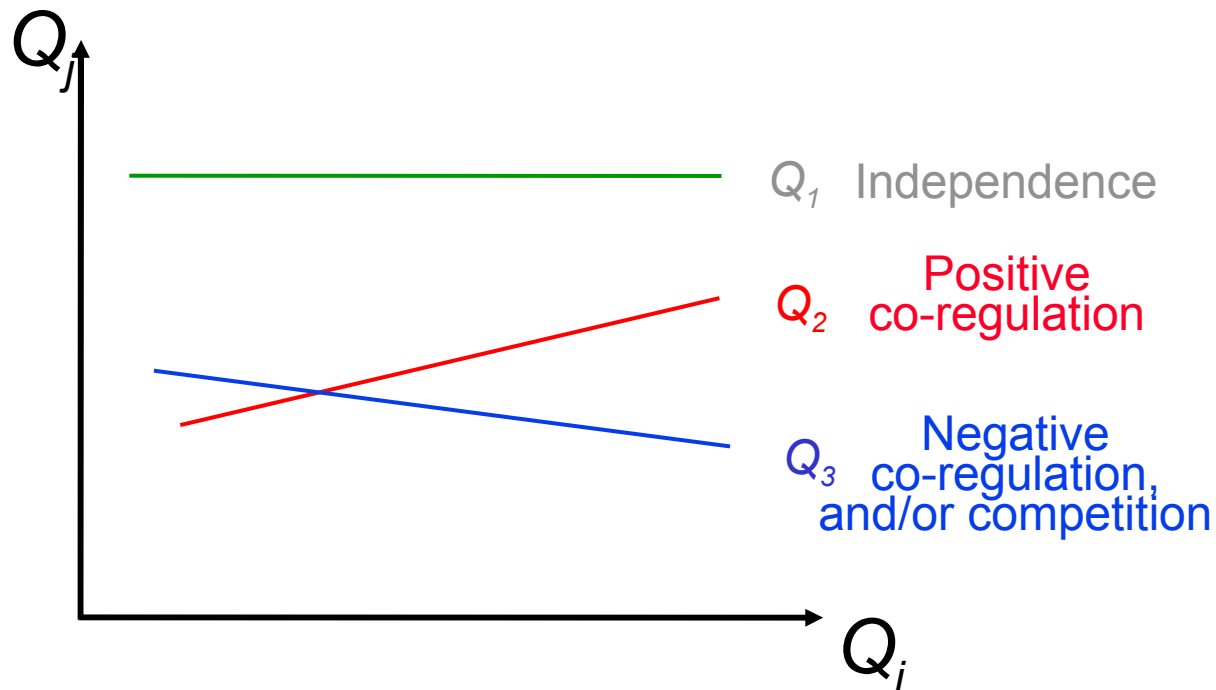


« Competition » between enzymes: concentrations are globally negatively correlated

Taking into account constraints on enzyme concentration II – Coregulation of gene expression

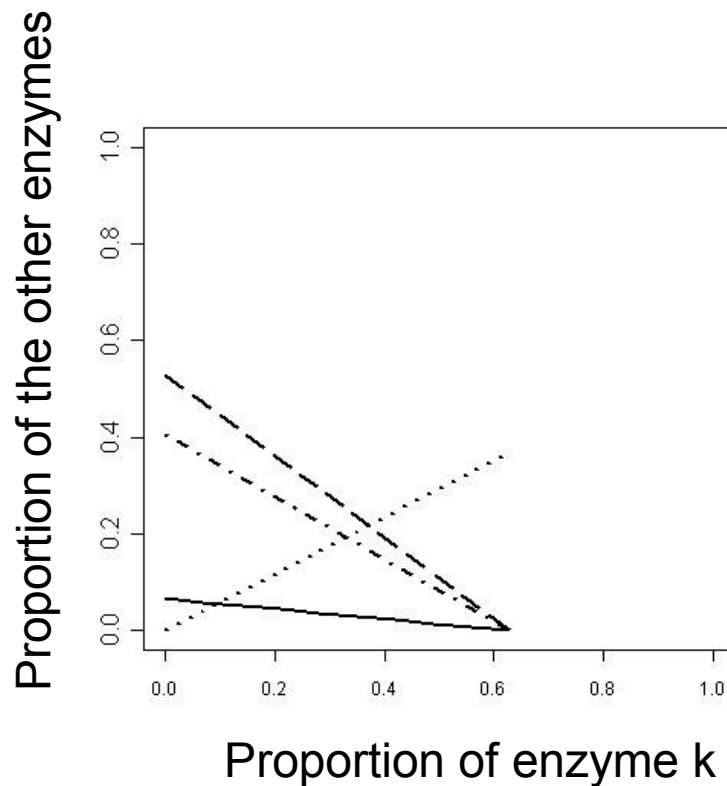
Two constraints on enzyme variation are considered,

Co-regulation *and/or* competition



Taking into account constraints on enzyme concentration II – Coregulation of gene expression

Co-regulation *and* competition



Redistribution
coefficient (slope):
$$\alpha_{ij} = \delta Q_j / \delta Q_i = -\beta_{ij} Q_j / (Q_{Tot} - Q_i)$$

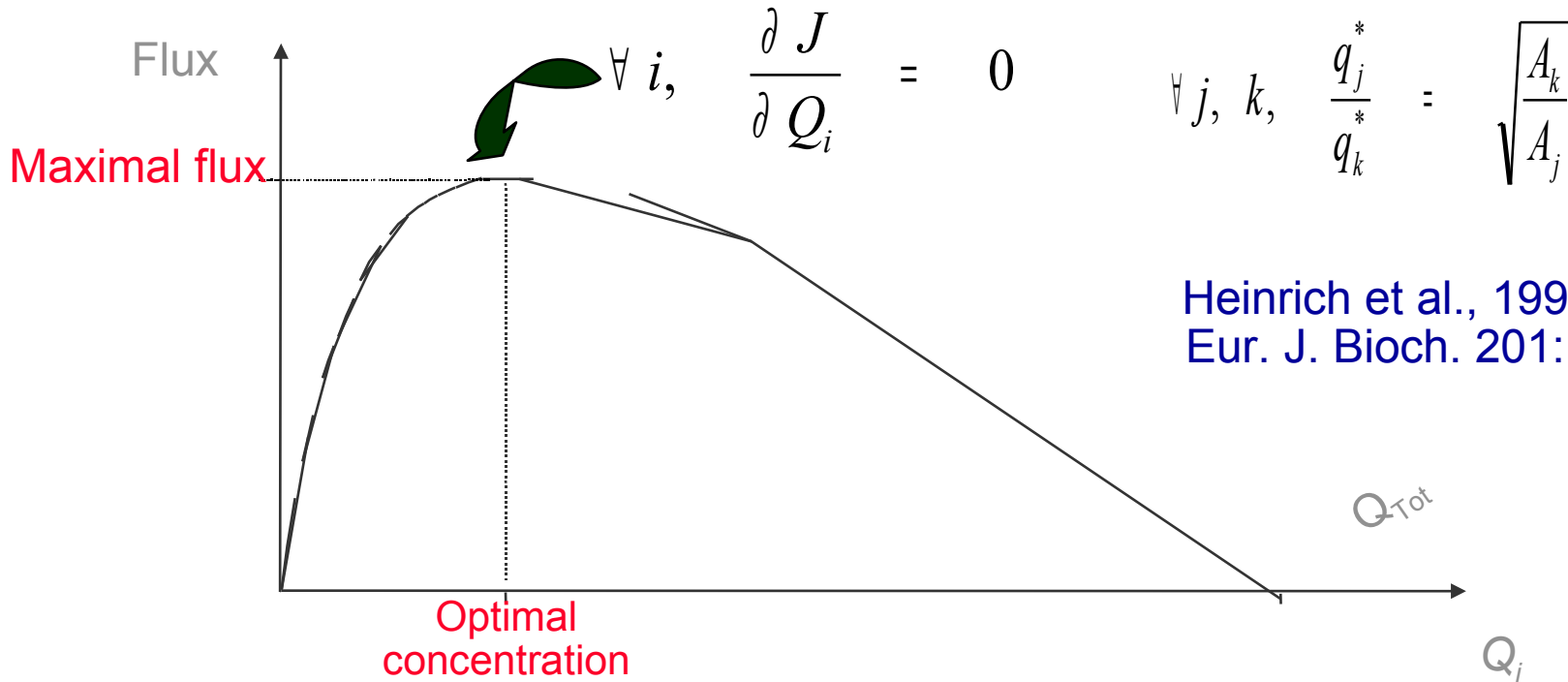
Can be positive or negative

Lion et al, 2004

Metabolic control theory with constraints on enzyme concentration

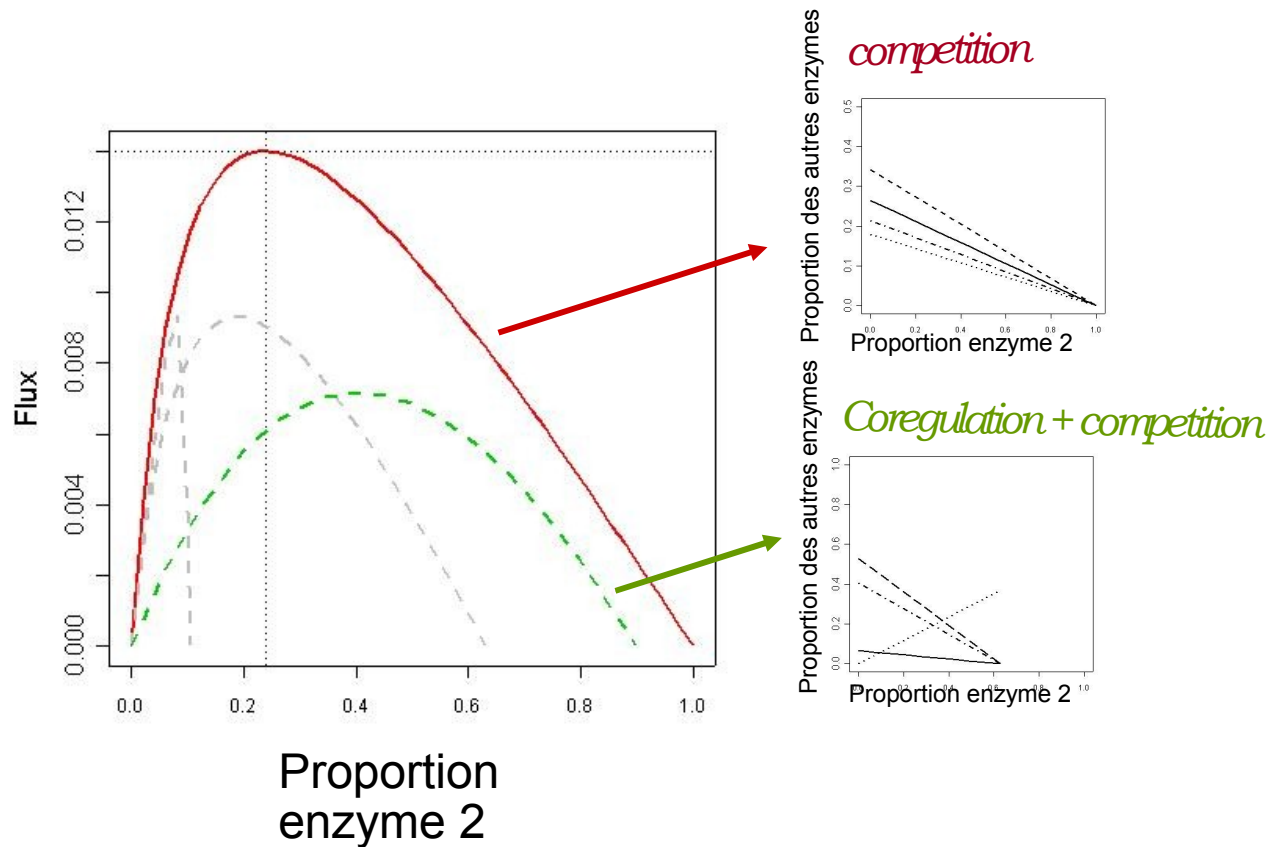
Optimal concentration of enzymes = the one which maximizes the flux ?

⇒ Lagrange's multiplier



Heinrich et al., 1991.
Eur. J. Bioch. 201:1.

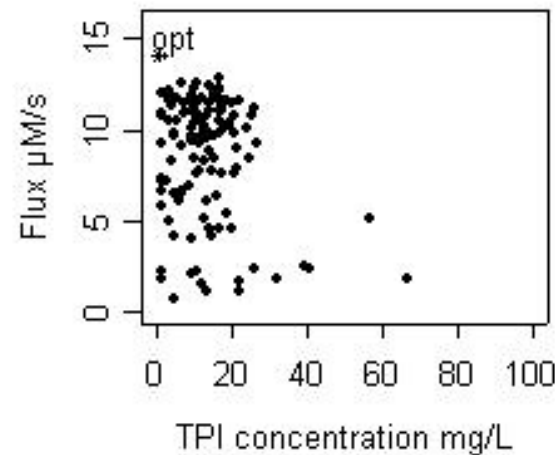
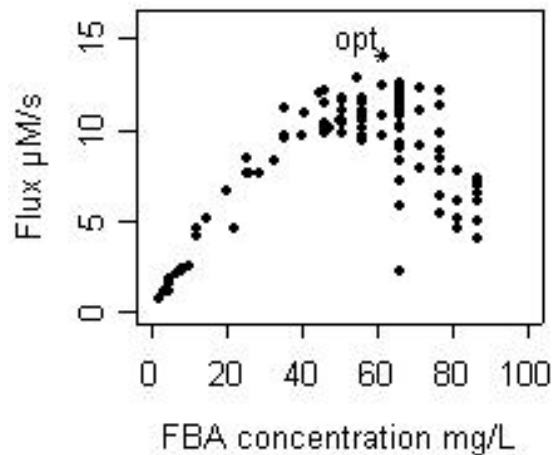
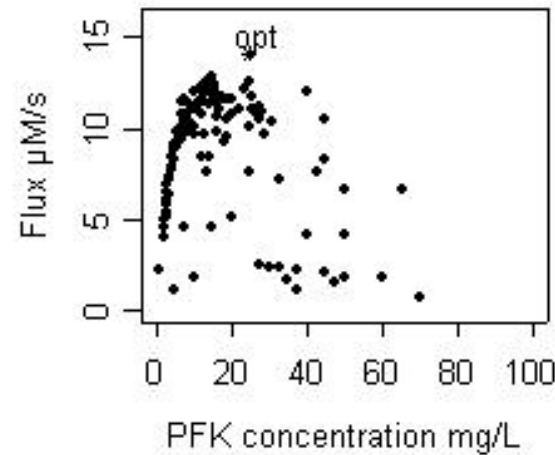
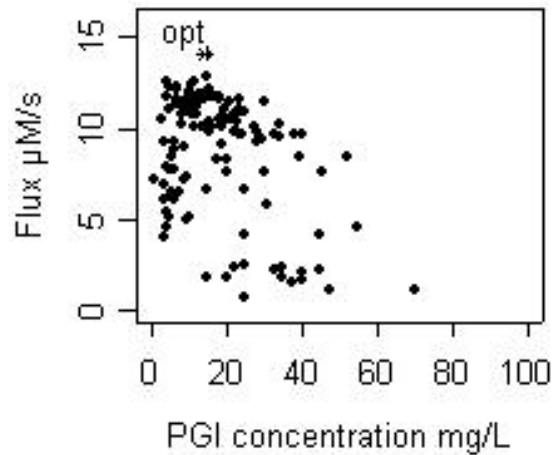
Metabolic control theory with constraints on enzyme concentration



- All experimental data points should be under the red curve
- At constant costs (Q_{tot}), maximal flux cannot be reached with co-regulations of enzyme concentrations

Lion et al, 2004

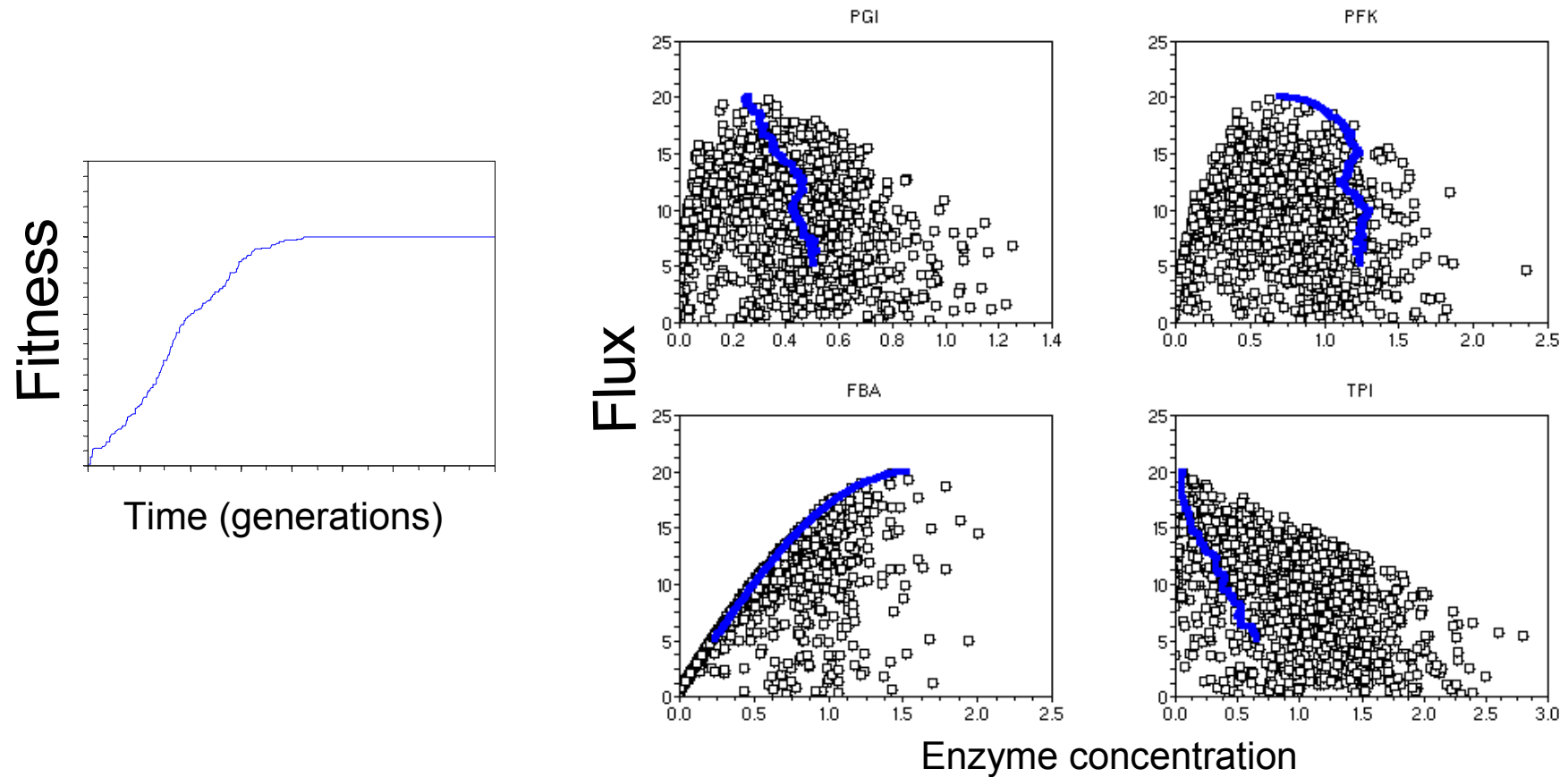
MCT with constraints on enzyme concentration: experimental validation



- All experimental data points should be under the red curve

Fievet et al, 2006

MCT with constraints: trajectories of enzyme concentrations during an adaptation bout



How does natural selection shape the exploration of the phenotypic space during adaptation ?