Are there recipes how to handle complexity?

Biological evolution creates complex entities and knows how to master them

Peter Schuster

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Complexity Primer

London, Law Society, 08.05.2008

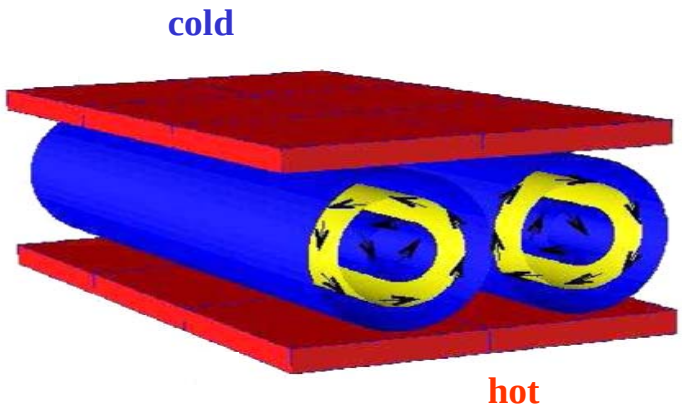
Web-Page for further information:

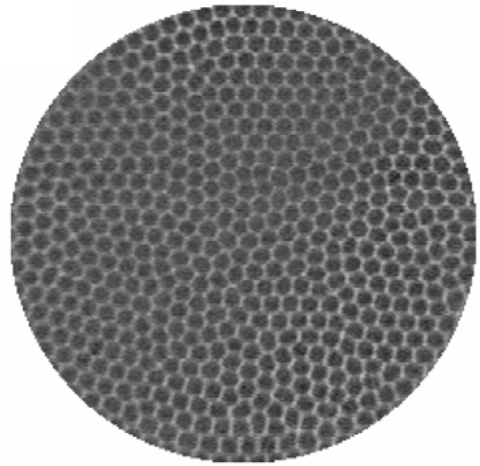
http://www.tbi.univie.ac.at/~pks

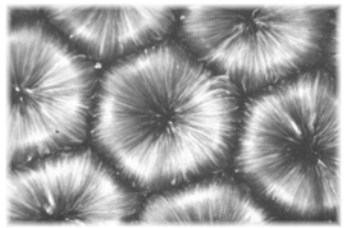
Catastrophic weather phenomena – strom, lightning, tornado and hurricane

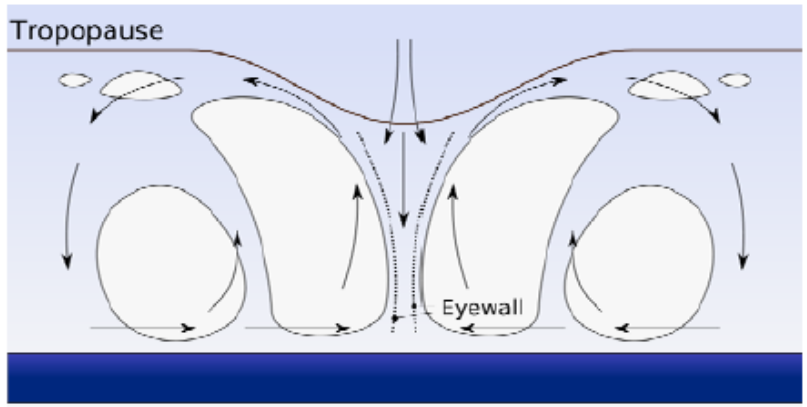
The Mayas of Chichen Itza

Pyramid, Chaac, and cenote sagrada









Raleigh-Bénard convection and hurricane formation

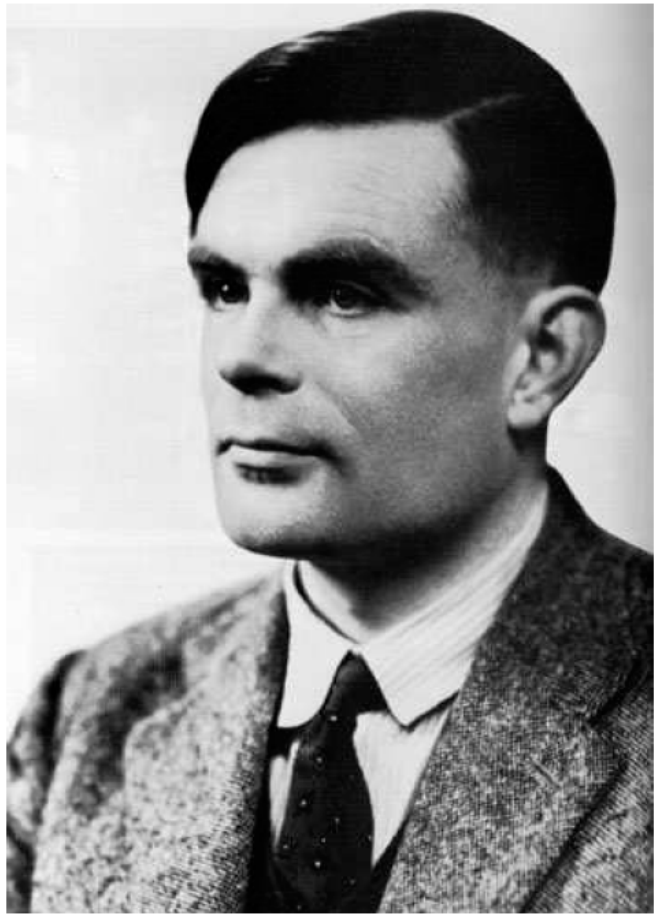
$$\frac{\partial u}{\partial t} = D_u \nabla^2 u + f(u,v)$$

$$\frac{\partial v}{\partial t} = D_v \nabla^2 v + g(u,v)$$

$$u = u(x,y,z,t) \quad \text{and} \quad v = v(x,y,z,t)$$

Change in local concentration =

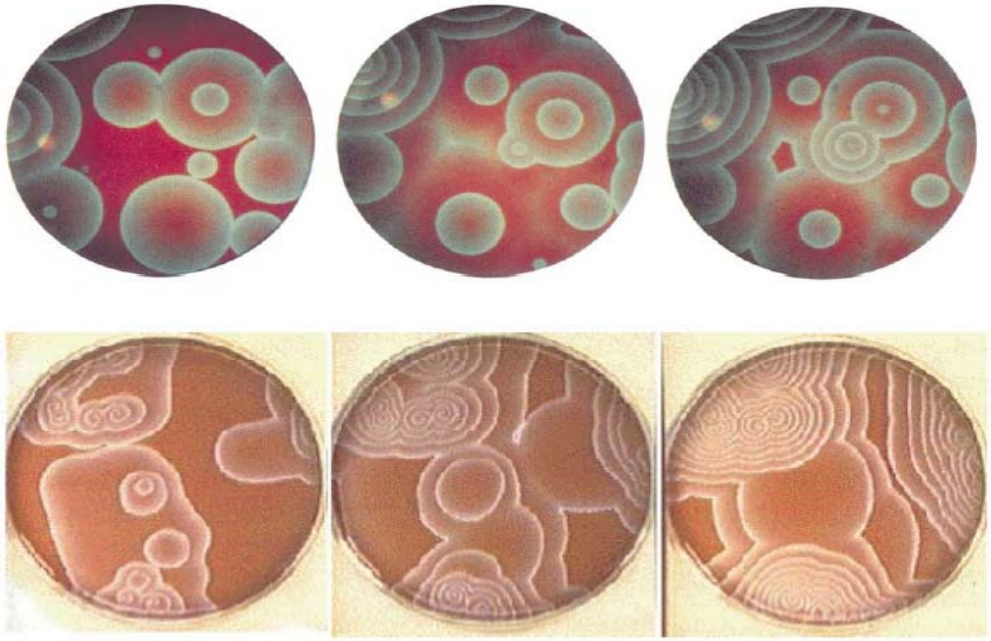
= diffusion + chemical reaction



Alan M. Turing, 1912-1954

A.M. Turing. 1952. The chemical basis of morphogenesis. *Phil.Trans.Roy.Soc.*London B **237**:37-72.

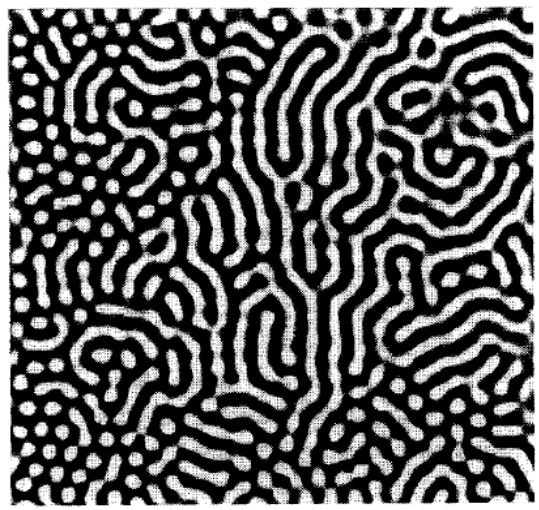
Liesegang rings 1895



Belousov-Zhabotinskii reaction 1959

Nonequilibrium patterns from chemical self-organization:

Liesegang rings in precipitation from oversaturated solutions, periodic patterns in the Belousov-Zhabotinskii reaction, and stationary Turing patterns.



Turing pattern:
Boissonade, De Kepper 1990

Color patterns on animal skins

Bates' mimicry

Müller's mimicry

Different forms of mimicry observed in nature

milk snake

Bates' mimicry

false coral snake

coral snake

Emsley's or Mertens' mimicry

Different forms of mimicry observed in nature

Skin patterns in an inbred strain of cats

Parents and daughter

Genotype, Genome

GCGGATTTAGCTCAGTTGGGAGAGCGCCAGACTGAAGATCTGGAGGTCCTGTGTTCGATCCACAGAATTCGCACCA

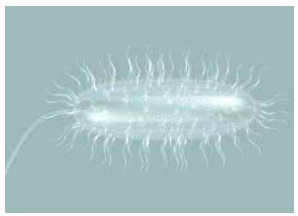
biochemistry
molecular biology
structural biology
molecular evolution
molecular genetics
systems biology
bioinfomatics

genetics
epigenetics
environment

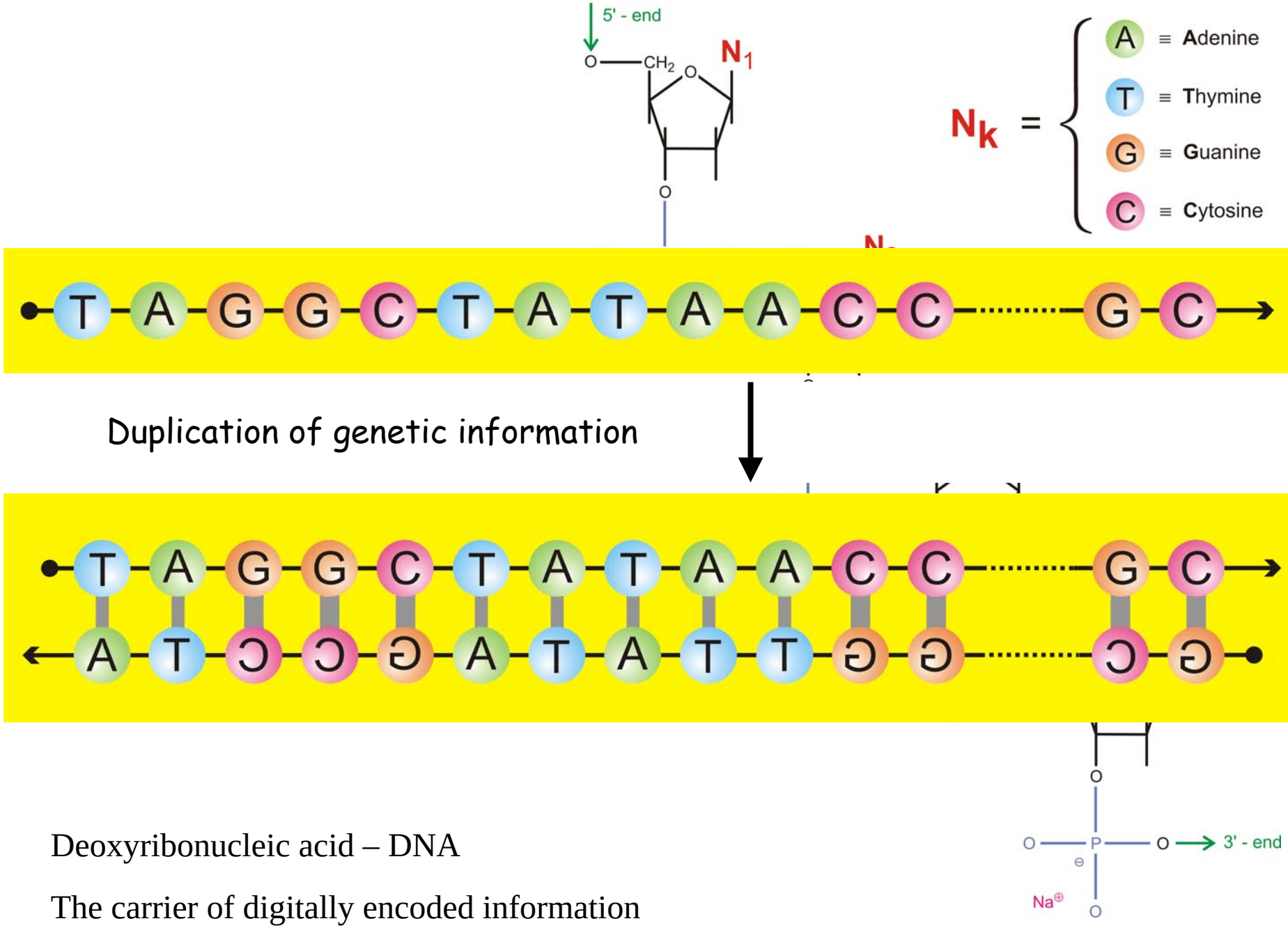
Unfolding of the genotype

development

cell biology
developmental biology
neurobiology
botany
zoology
anthropology
ecology

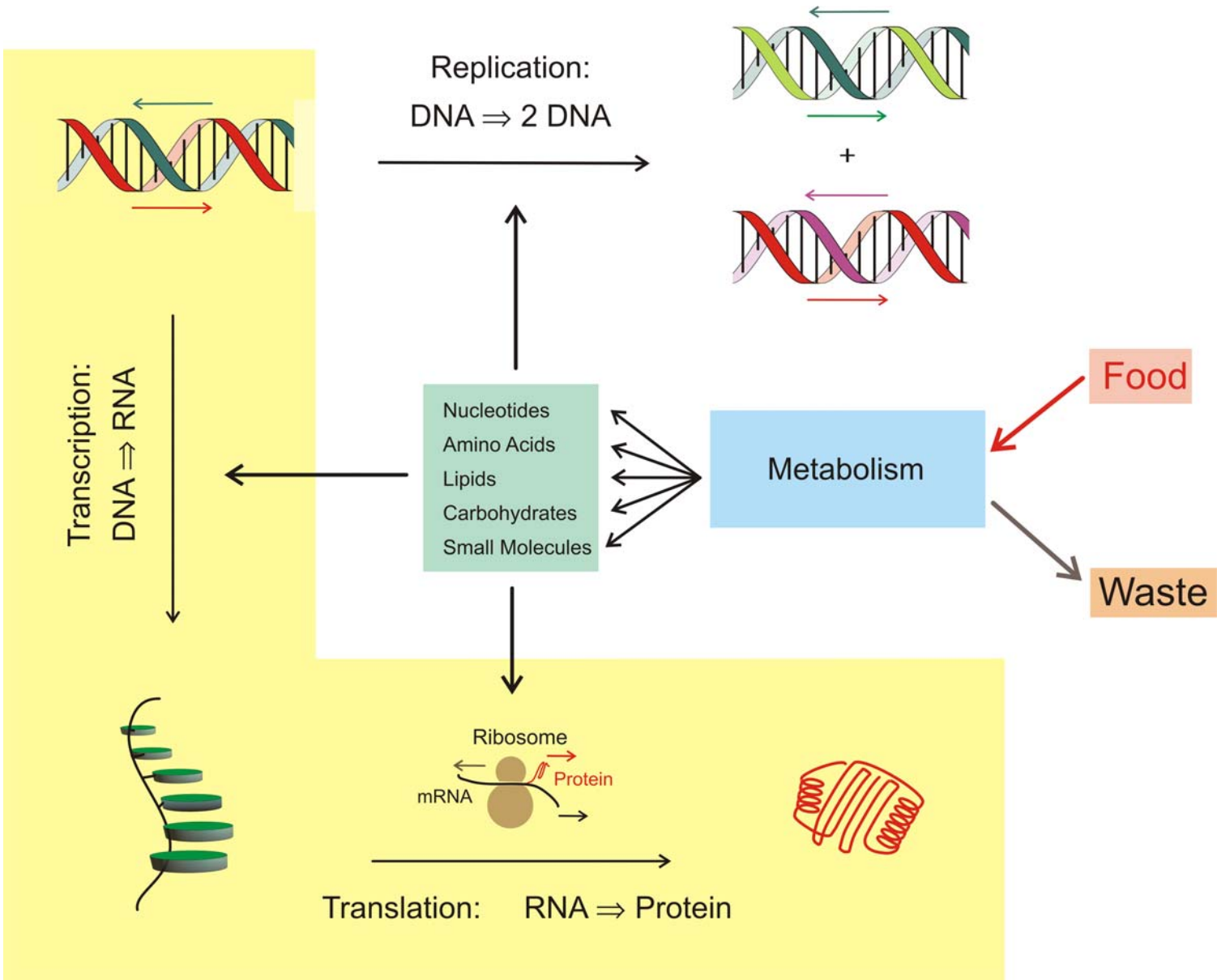


Phenotype

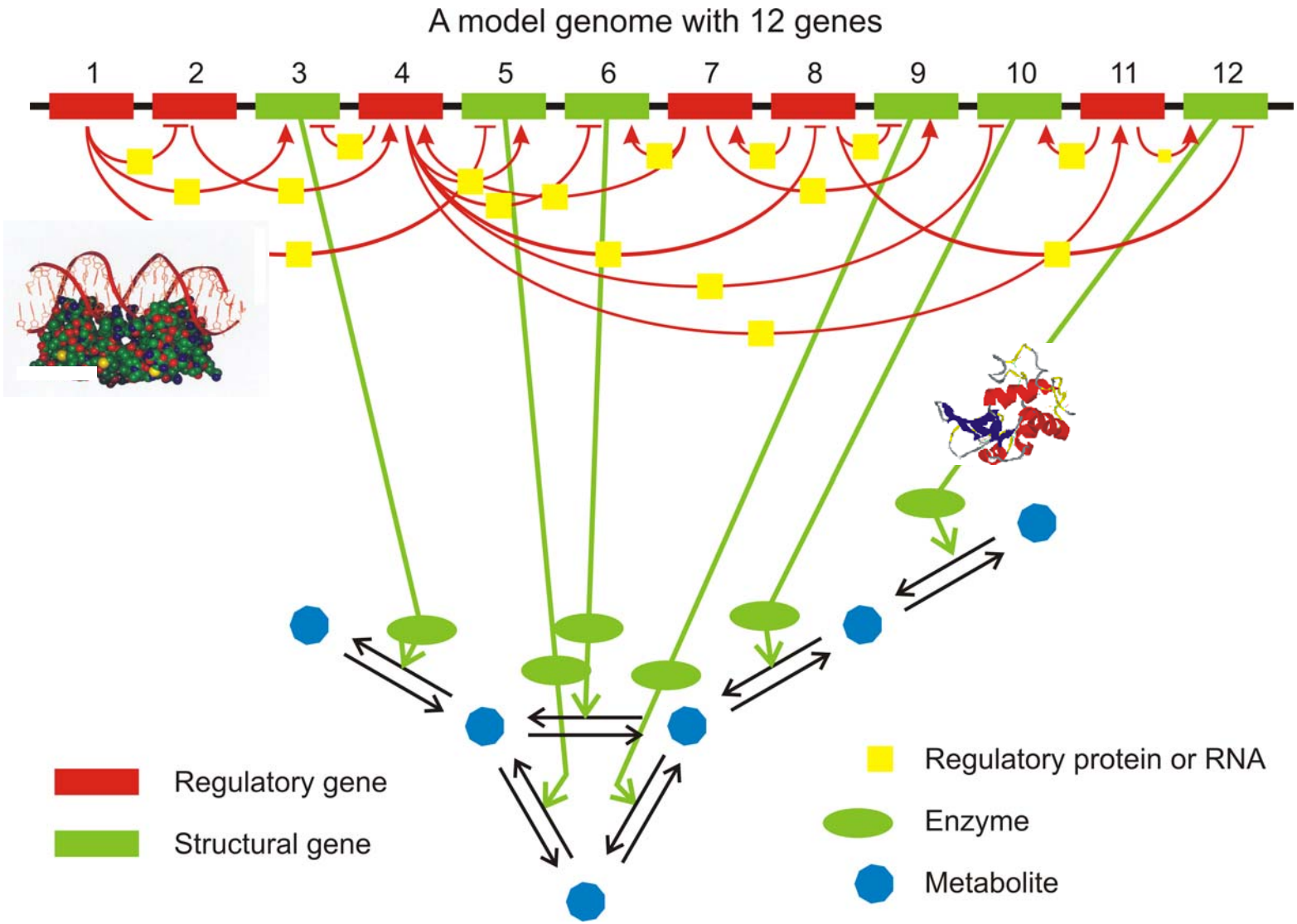


Deoxyribonucleic acid – DNA

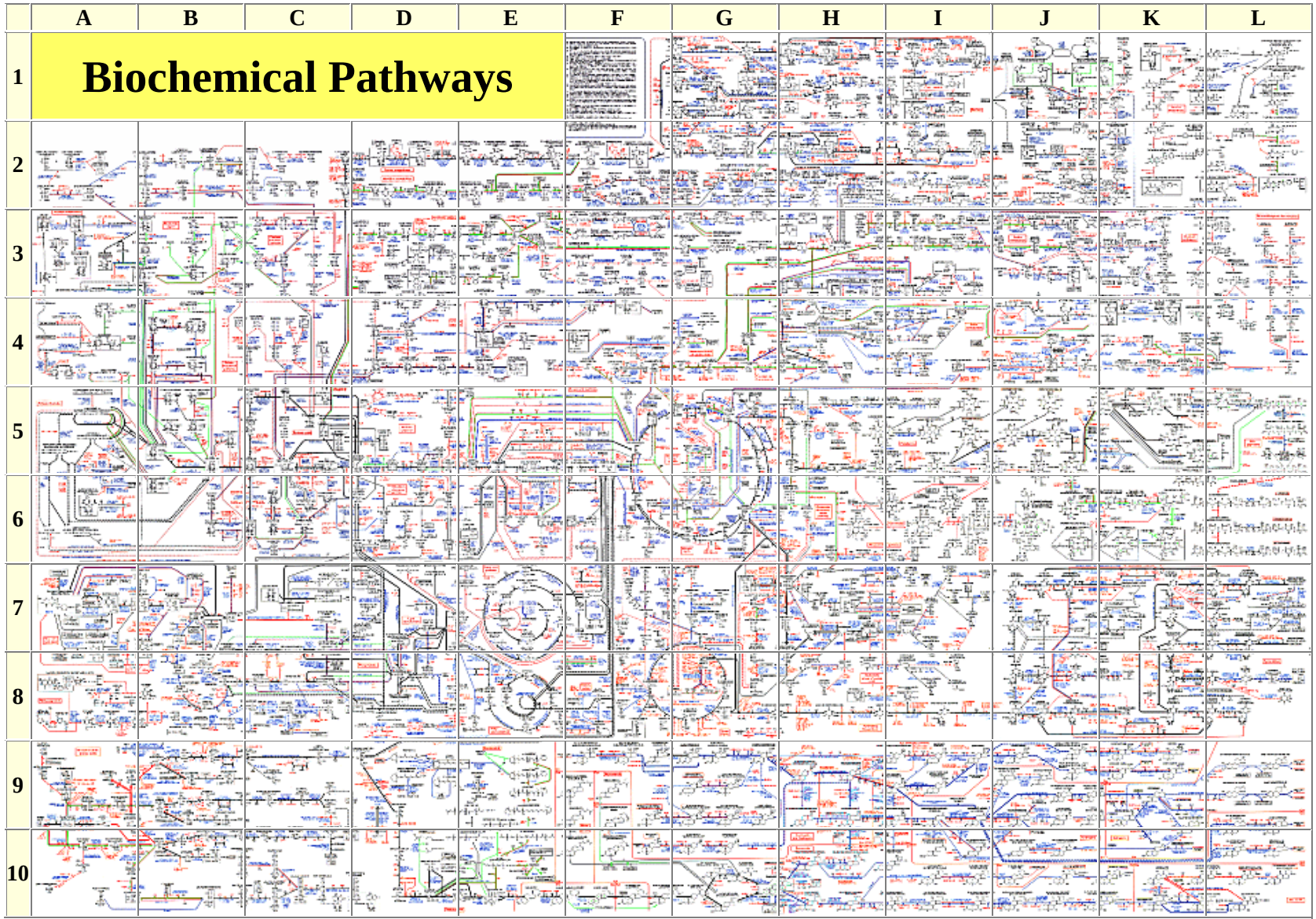
The carrier of digitally encoded information



A sketch of cellular information processing

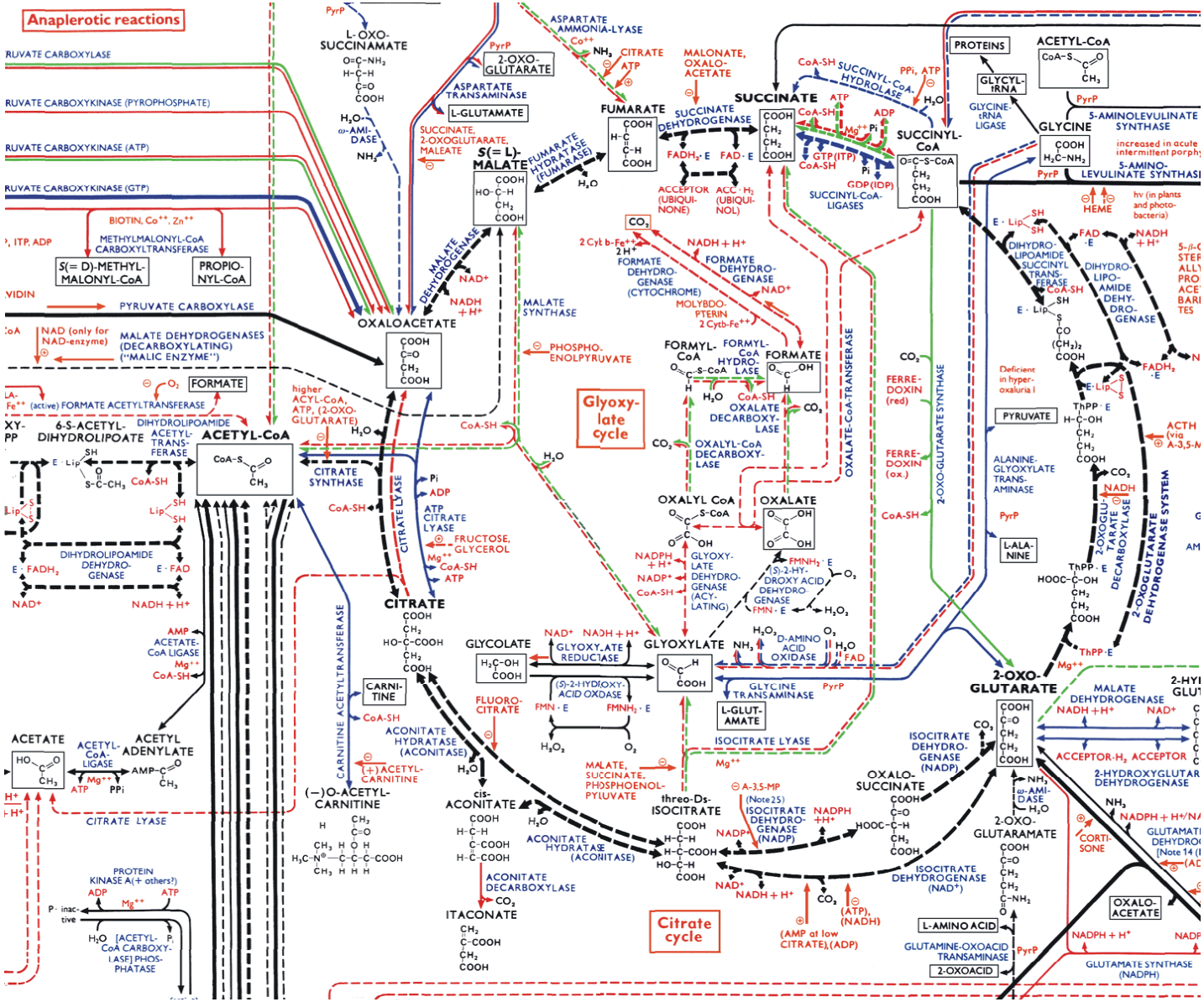


A sketch of a genetic and metabolic network



The reaction network of cellular metabolism published by Boehringer-Ingelheim.

The citric acid or Krebs cycle (enlarged from previous slide).



Three necessary conditions for Darwinian evolution are:

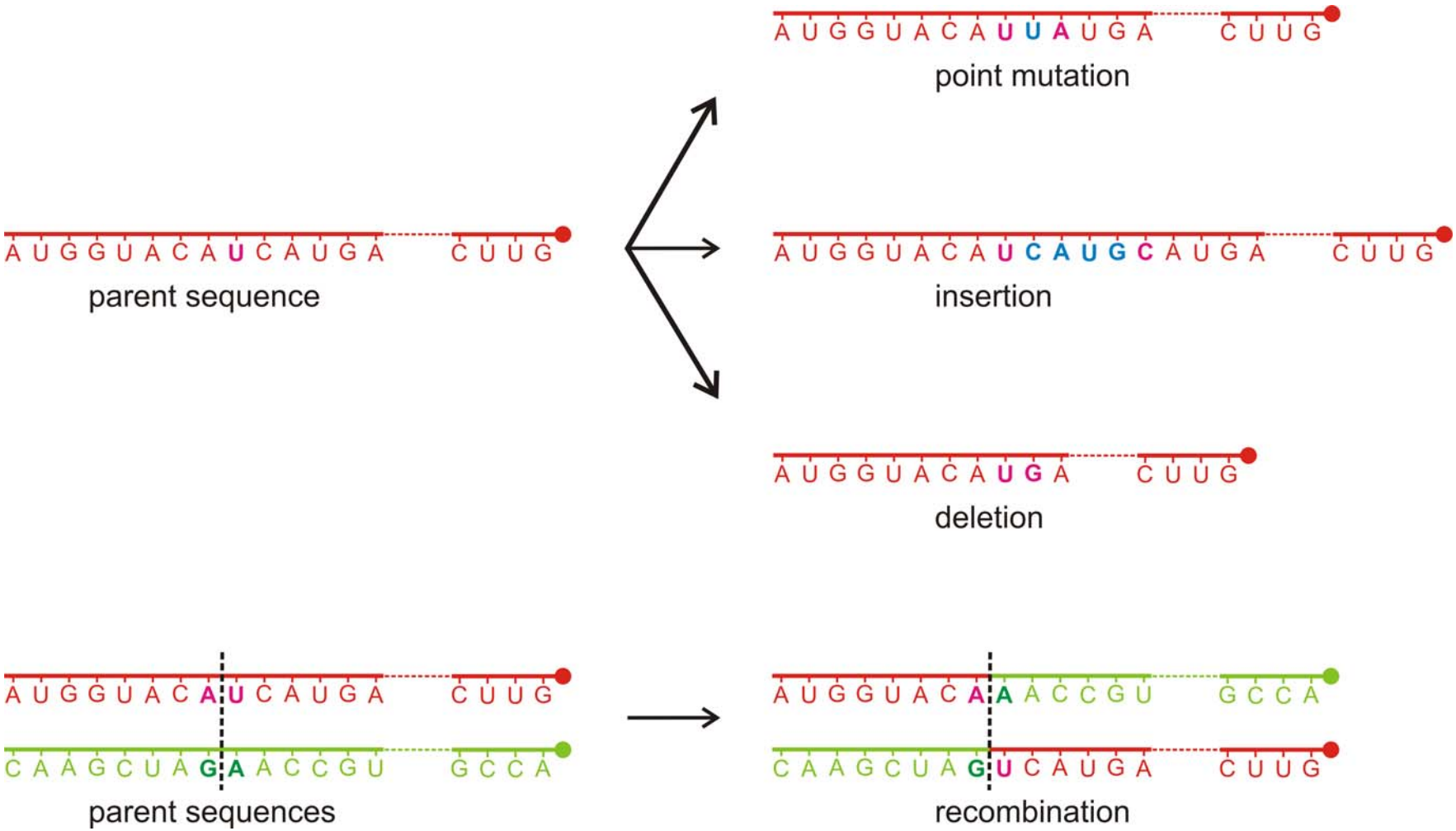
1. **Multiplication,**
2. **Variation**, and
3. **Selection**.

Multiplication is a basic property of all cells in germ lines.

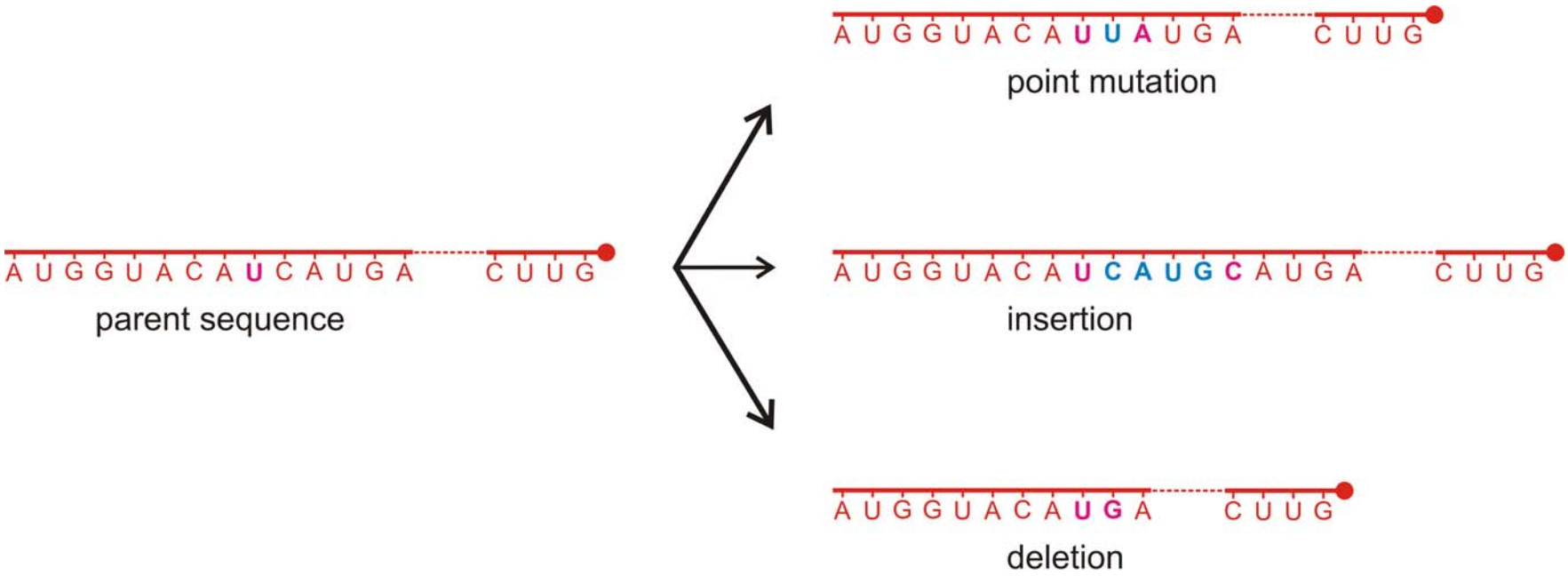
Variation through mutation and recombination operates on the **genotype** whereas the **phenotype** is the target of **selection**. **Variations**, mutations or recombination events, occur **uncorrelated** with their effects on the **selection** process.

Selection is a consequence of finite population sizes.

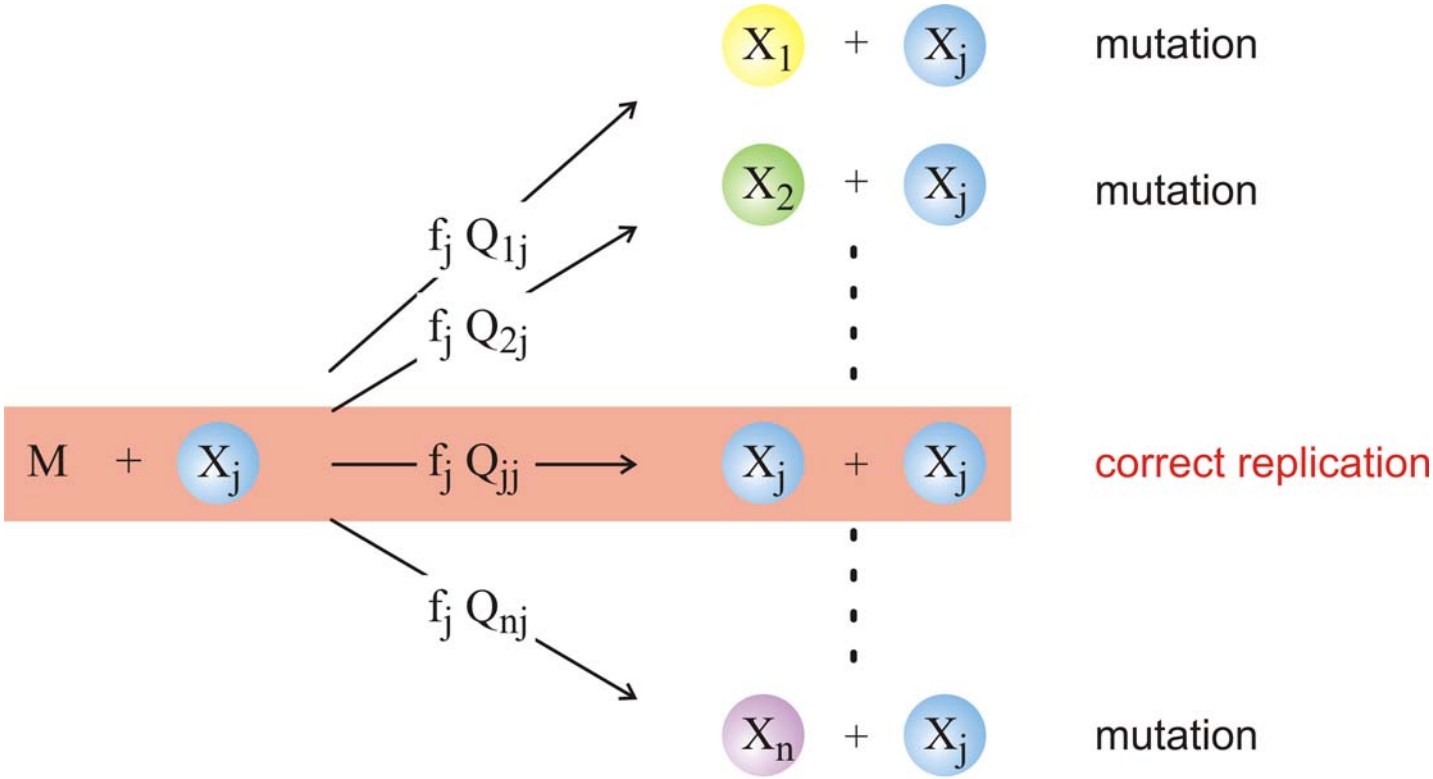
All conditions can be fulfilled not only by cellular organisms but also by nucleic acid molecules in suitable cell-free experimental assays.



Variation of genotypes through mutation and recombination



Variation of genotypes through mutation



f_j ... replication rate function or fitness function

Q_{ij} ... mutation frequency: $X_j \rightarrow X_i$

Chemical kinetics of molecular evolution

M. Eigen, P. Schuster, `The Hypercycle´, Springer-Verlag, Berlin 1979

$$\frac{dx_i}{dt} = \sum_{j=1}^{N} Q_{ij}\, f_j(\mathbf{x})\, x_j \;-\; x_i\, \Phi(\mathbf{x})\,, \quad i = 1, 2, \ldots, N$$

$$\Phi(\mathbf{x}) = \sum_{j=1}^{N} f_j(\mathbf{x})\, x_j\,, \quad \sum_{j=1}^{n} x_j = 1\,, \quad \text{and} \quad N = 4^n$$

Chemical kinetics of molecular evolution

M. Eigen, P. Schuster, `The Hypercycle´, Springer-Verlag, Berlin 1979

DIE NATURWISSENSCHAFTEN

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Selforganization of Matter and the Evolution of Biological Macromolecules

MANFRED EIGEN*

Max-Planck-Institut für Biophysikalische Chemie, Karl-Friedrich-Bonhoeffer-Institut, Göttingen-Nikolausberg

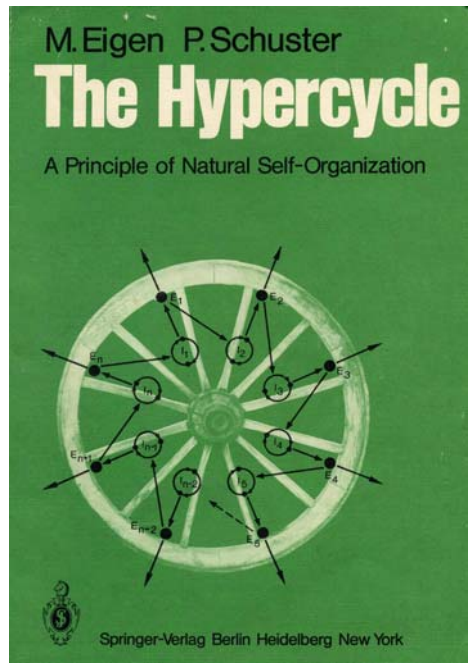
I. Introduction

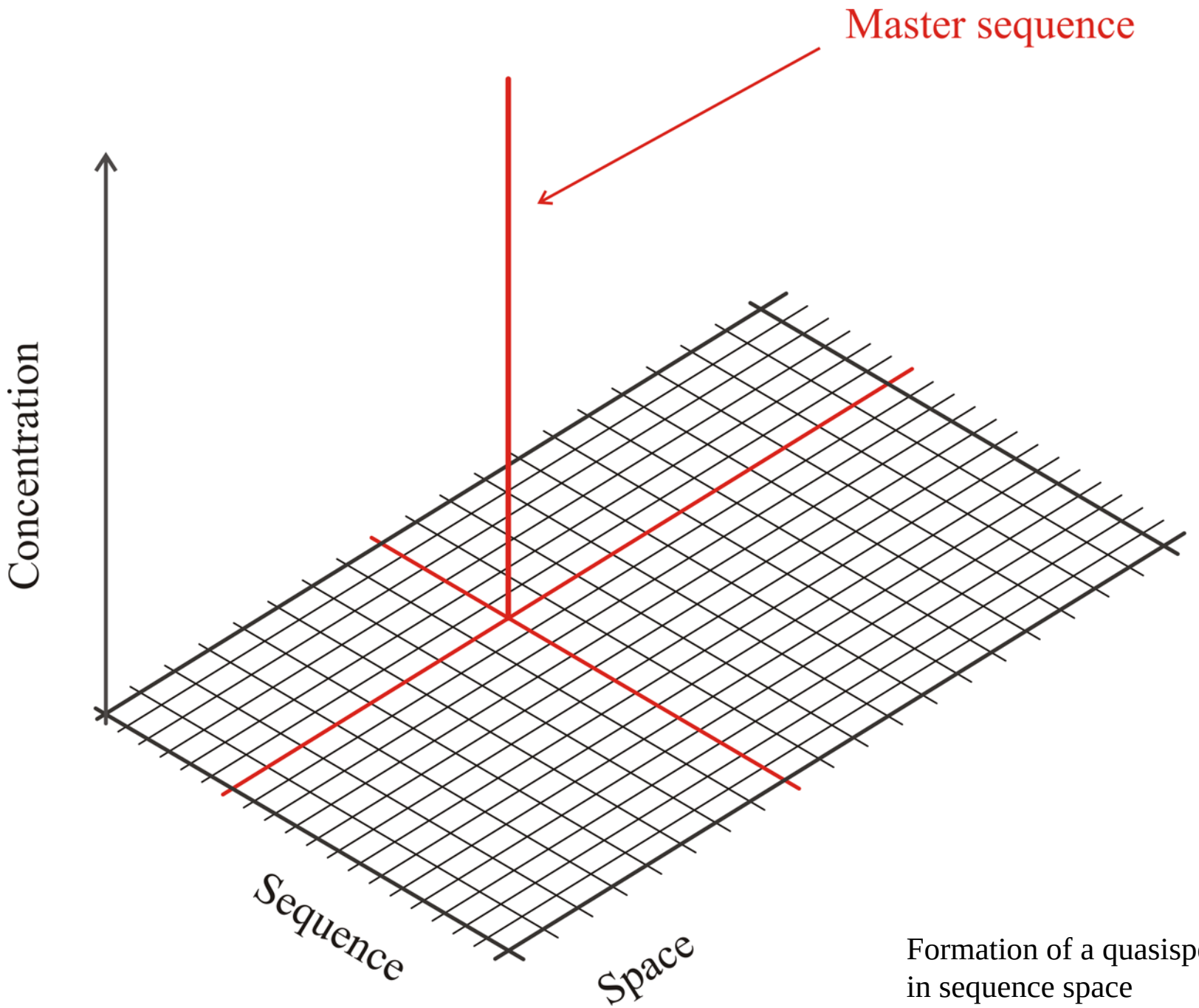
I.1. „Cause and Effect"

The question about the origin of life often appears as a question about "cause and effect". Physical theories of macroscopic processes usually involve answers to such questions, even if a statistical interpretation is given to the relation between "cause" and "effect". It is mainly due to the nature of this question that many scientists believe that our present physics does not offer any obvious explanation for the existence of life, which even in its simplest forms always appears to be associated with complex macroscopic (i.e. multimolecular) systems, such as the living cell.

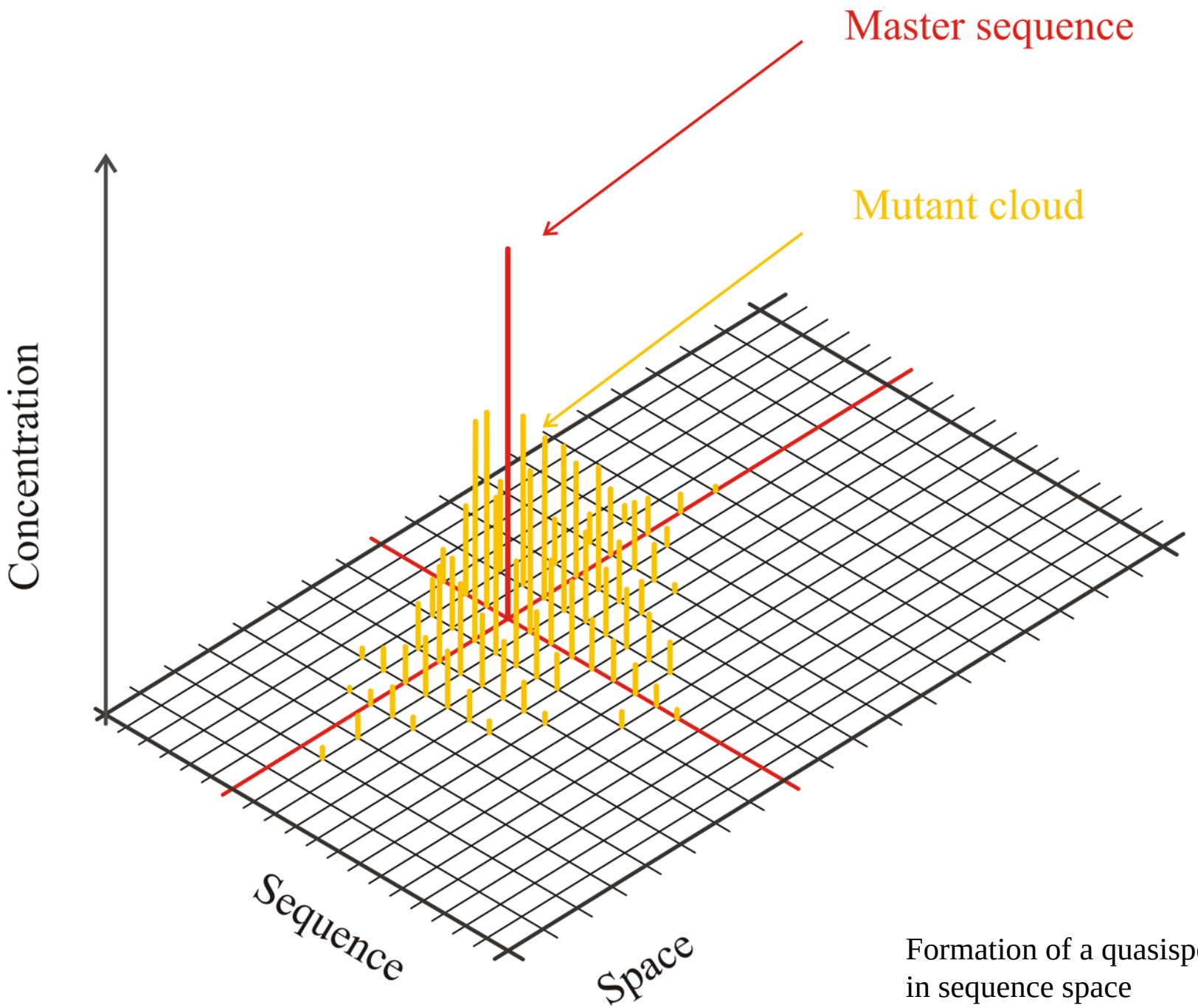
As a consequence of the exciting discoveries of "molecular biology", a common version of the above question is: *Which came first, the protein or the nucleic acid?* – a modern variant of the old "chicken-and-egg" problem. The term "first" is usually meant to define a causal rather than a temporal relationship, and the words "protein" and "nucleic acid" may be substituted by "function" and "information". The question in this form, when applied to the interplay of nucleic acids and proteins as presently encountered in the living cell, leads ad absurdum, because "function"

* Partly presented as the "Robbins Lectures" at Pomona College, California, in spring 1970.





Formation of a quasispecies in sequence space



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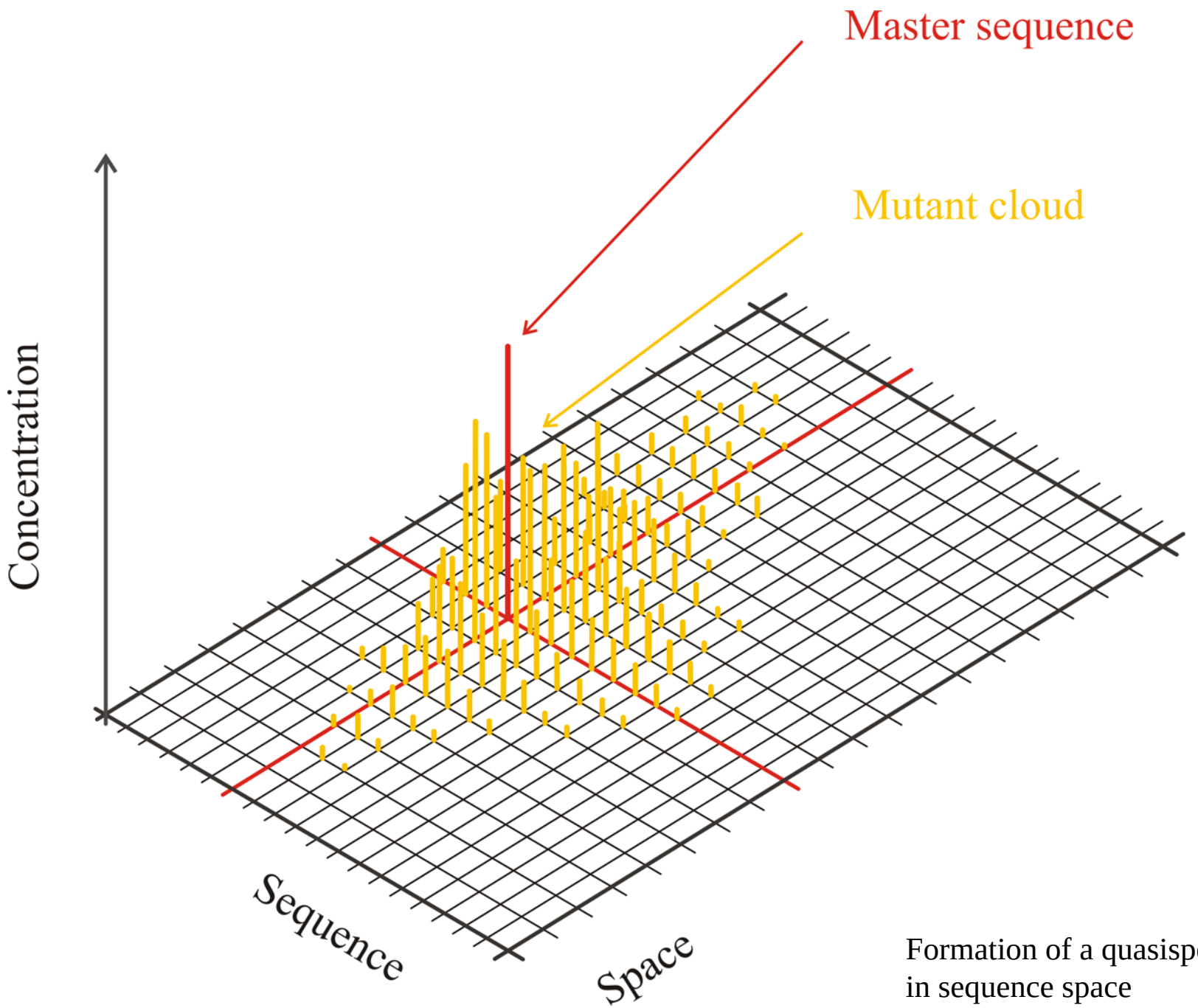

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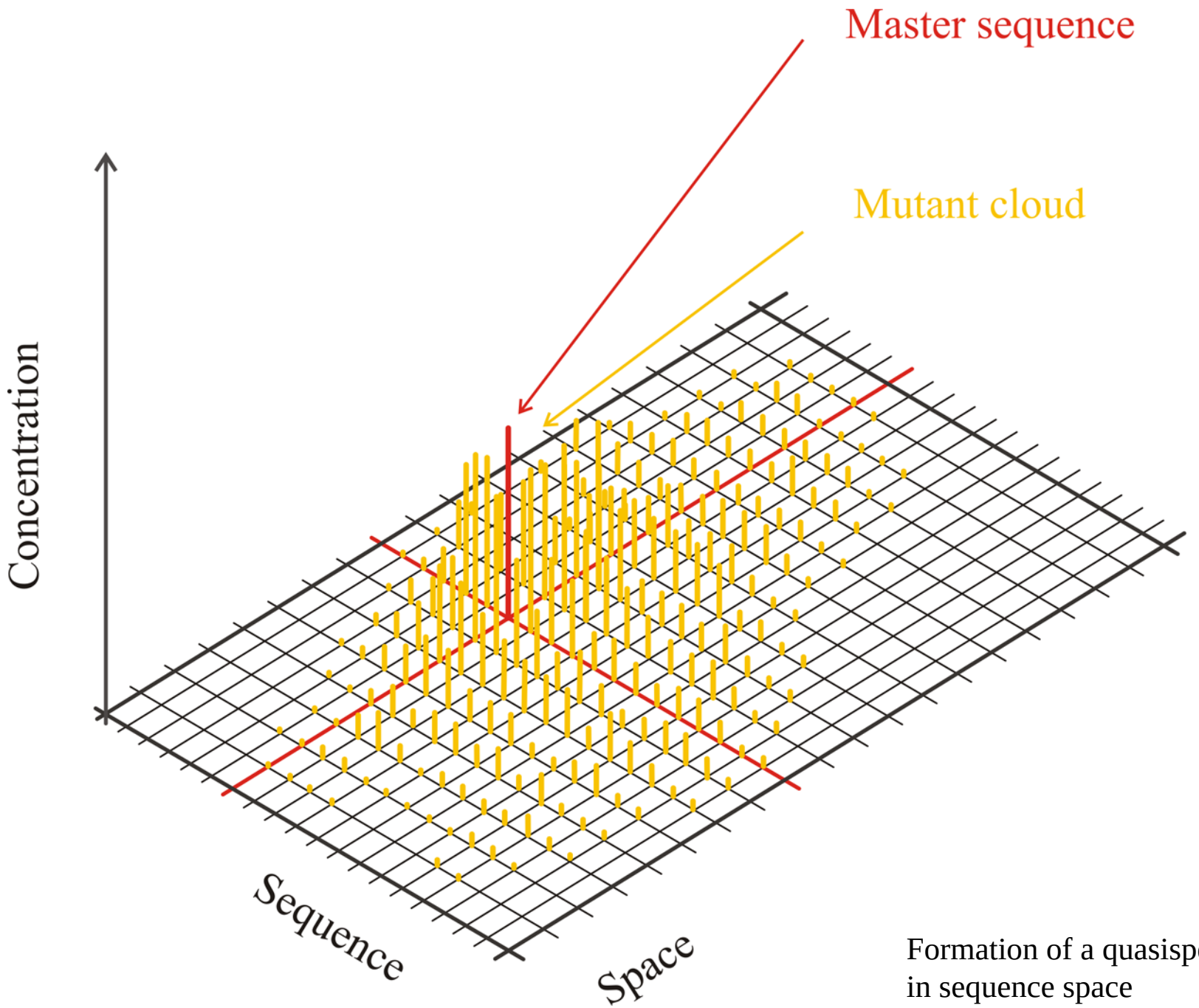
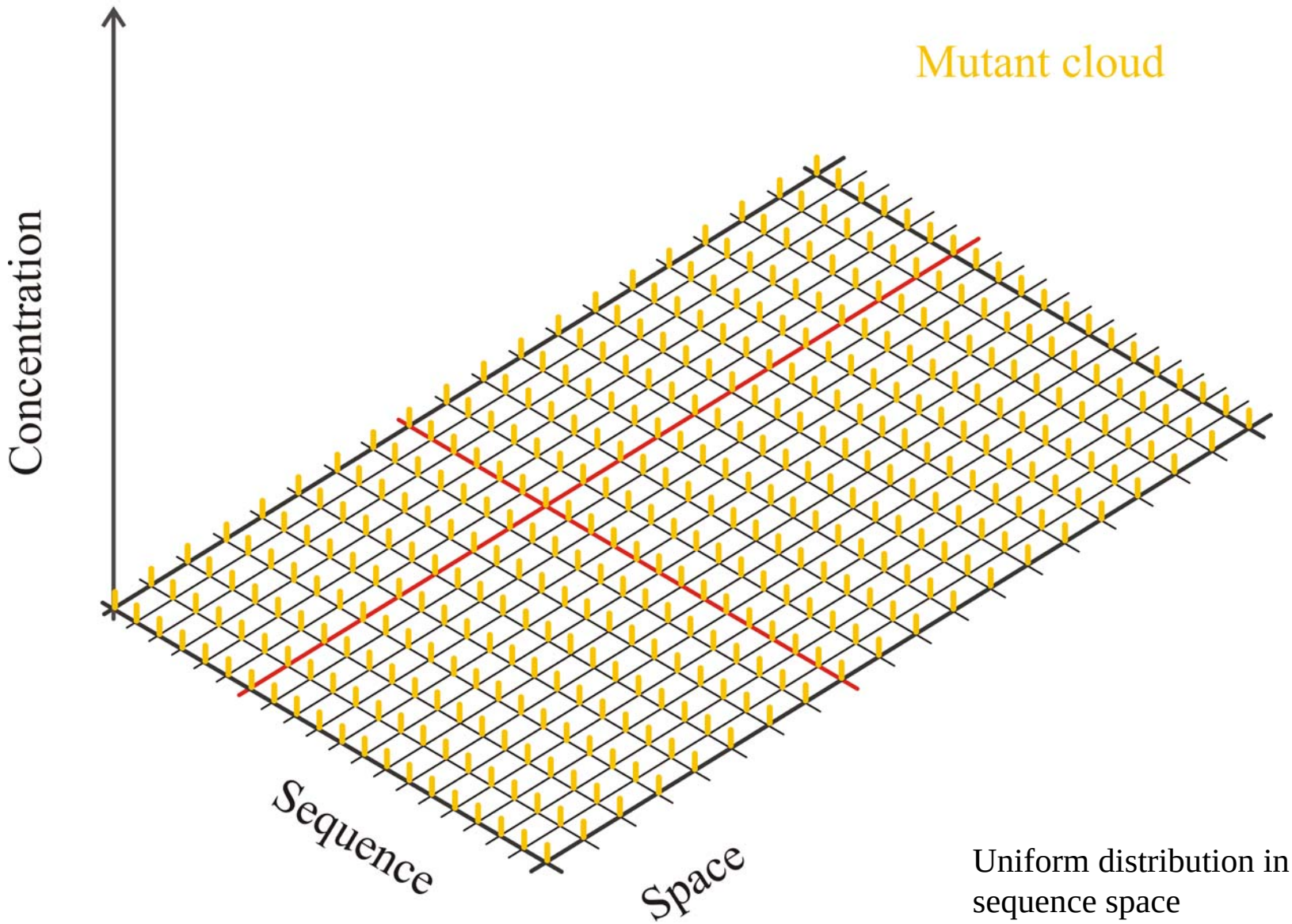
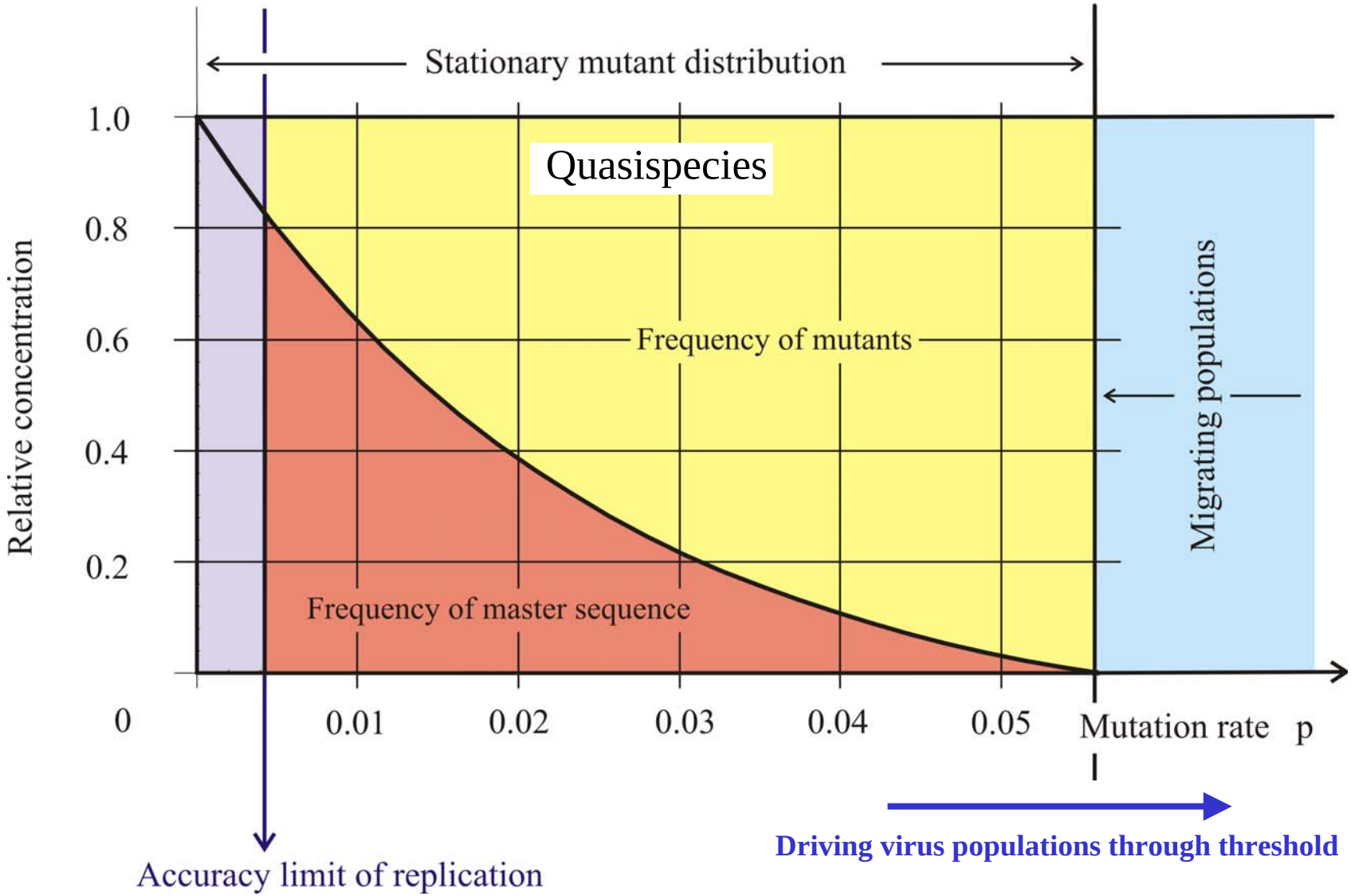


Formation of a quasispecies in sequence space



Uniform distribution in sequence space



The error threshold in replication

Available online at www.sciencedirect.com
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Virus Research 107 (2005) 115–116
Virus Research
www.elsevier.com/locate/virusres

Preface

Antiviral strategy on the horizon

Error catastrophe had its conceptual origins in the middle of the XXth century, when the consequences of mutations on enzymes involved in protein synthesis, as a theory of aging. In those times biological processes were generally perceived differently from today. Infectious diseases were regarded as a fleeting nuisance which would be eliminated through the use of antibiotics and antiviral agents. Microbial variation, although known in some cases, was not thought to be a significant problem for disease control. Variation in differentiated organisms was seen as resulting essentially from exchanges of genetic material associated with sexual reproduction. The problem was to unveil the mechanisms of inheritance, expression of genetic information and metabolism. Few saw that genetic change is occurring at present in all organisms, and still fewer recognized Darwinian principles as essential to the biology of pathogenic viruses and cells. Population geneticists rarely used bacteria or viruses as experimental systems to define concepts in biological evolution. The extent of genetic polymorphism among individuals of the same biological species came as a surprise when the first results on comparison of electrophoretic mobility of enzymes were obtained. With the advent of in vitro DNA recombination, and rapid nucleic acid sequencing techniques, molecular analyses of genomes reinforced the conclusion of extreme inter-individual genetic variation within the same species. Now, due largely to spectacular progress in comparative genomics, we see cellular DNAs, both prokaryotic and eukaryotic, as highly dynamic. Most cellular processes, including such essential information-bearing and transferring events as genome replication, transcription and translation, are increasingly perceived as inherently inaccurate. Viruses, and in particular RNA viruses, are among the most extreme examples of exploitation of replication inaccuracy for survival.

Error catastrophe, or the loss of meaningful genetic information through excess genetic variation, was formulated in quantitative terms as a consequence of quasispecies theory, which was first developed to explain self-organization and adaptability of primitive replicons in early stages of life. Recently, a conceptual extension of error catastrophe that could be defined as "induced genetic deterioration" has emerged as a possible antiviral strategy. This is the topic of the current special issue of ***Virus Research***.

Few would nowadays doubt that one of the major obstacles for the control of viral disease is short-term adaptability of viral pathogens. Adaptability of viruses follows the same Darwinian principles that have shaped biological evolution over eons, that is, repeated rounds of reproduction with genetic variation, competition and selection, often perturbed by random events such as statistical fluctuations in population size. However, with viruses the consequences of the operation of these very same Darwinian principles are felt within very short times. Short-term evolution (within hours and days) can be also observed with some cellular pathogens, with subsets of normal cells, and cancer cells. The nature of RNA viral pathogens begs for alternative antiviral strategies, and forcing the virus to cross the critical error threshold for maintenance of genetic information is one of them.

The contributions to this volume have been chosen to reflect different lines of evidence (both theoretical and experimental) on which antiviral designs based on genetic deterioration inflicted upon viruses are being constructed. Theoretical studies have explored the copying fidelity conditions that must be fulfilled by any information-bearing replication system for the essential genetic information to be transmitted to progeny. Closely related to the theoretical developments have been numerous experimental studies on quasispecies dynamics and their multiple biological manifestations. The latter can be summarized by saying that RNA viruses, by virtue of existing as mutant spectra rather than defined genetic entities, remarkably expand their potential to overcome selective pressures intended to limit their replication. Indeed, the use of antiviral inhibitors in clinical practice and the design of vaccines for a number of major RNA virus-associated diseases, are currently presided by a sense of uncertainty. Another line of growing research is the enzymology of copying fidelity by viral replicases, aimed at understanding the molecular basis of mutagenic activities. Error catastrophe as a potential new antiviral strategy received an important impulse by the observation that ribavirin (a licensed antiviral nucleoside analogue) may be exerting, in some systems, its antiviral activity through enhanced mutagenesis. This has encouraged investigations on new mutagenic base analogues, some of them used in anticancer chemotherapy. Some chapters summarize these important biochemical studies on cell entry pathways and metabolism of mutagenic agents, that may find new applications as antiviral agents.

This volume intends to be basically a progress report, an introduction to a new avenue of research, and a realistic appraisal of the many issues that remain to be investigated. In this respect, I can envisage (not without many uncertainties) at least three lines of needed research: (i) One on further understanding of quasispecies dynamics in infected individuals to learn more on how to apply combinations of virus-specific mutagens and inhibitors in an effective way, finding synergistic combinations and avoiding antagonistic ones as well as severe clinical side effects. (ii) Another on a deeper understanding of the metabolism of mutagenic agents, in particular base and nucleoside analogues. This includes identification of the transporters that carry them into cells, an understanding of their metabolic processing, intracellular stability and alterations of nucleotide pools, among other issues. (iii) Still another line of needed research is the development of new mutagenic agents specific for viruses, showing no (or limited) toxicity for cells. Some advances may come from links with anticancer research, but others should result from the designs of new molecules, based on the structures of viral polymerases. I really hope that the reader finds this issue not only to be an interesting and useful review of the current situation in the field, but also a stimulating exposure to the major problems to be faced.

The idea to prepare this special issue came as a kind invitation of Ulrich Desselberger, former Editor of ***Virus Research***, and then taken enthusiastically by Luis Enjuanes, recently appointed as Editor of ***Virus Research***. I take this opportunity to thank Ulrich, Luis and the Editor-in-Chief of ***Virus Research***, Brian Mahy, for their continued interest and support to the research on virus evolution over the years.

My thanks go also to the 19 authors who despite their busy schedules have taken time to prepare excellent manuscripts, to Elsevier staff for their prompt responses to my requests, and, last but not least, to Ms. Lucía Horrillo from Centro de Biología Molecular "Severo Ochoa" for her patient dealing with the correspondence with authors and the final organization of the issue.

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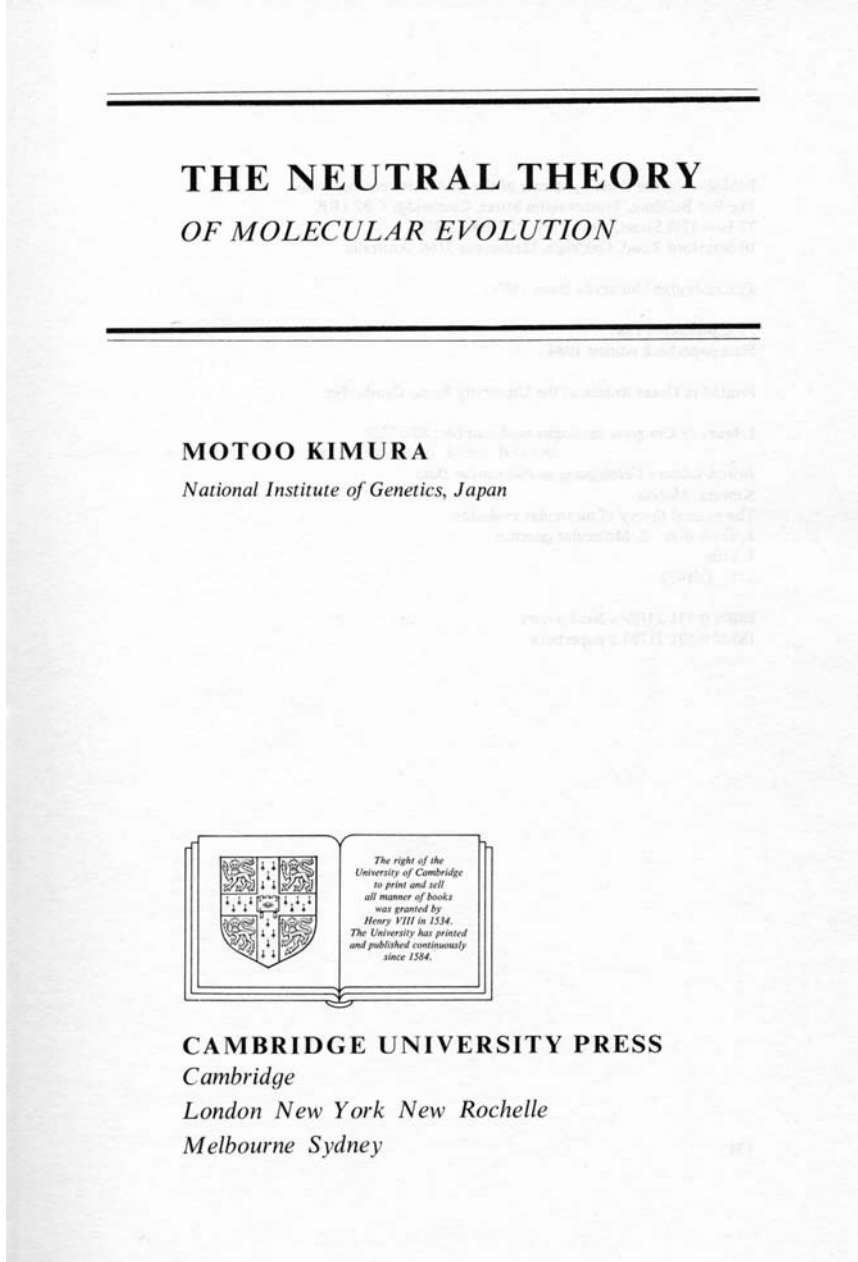
MOTOO KIMURA

Motoo Kimuras Populationsgenetik der neutralen Evolution.

Evolutionary rate at the molecular level. *Nature* **217**: 624-626, 1955.

The Neutral Theory of Molecular Evolution. Cambridge University Press. Cambridge, UK, 1983.



THE NEUTRAL THEORY

OF MOLECULAR EVOLUTION

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National Institute of Genetics, Japan

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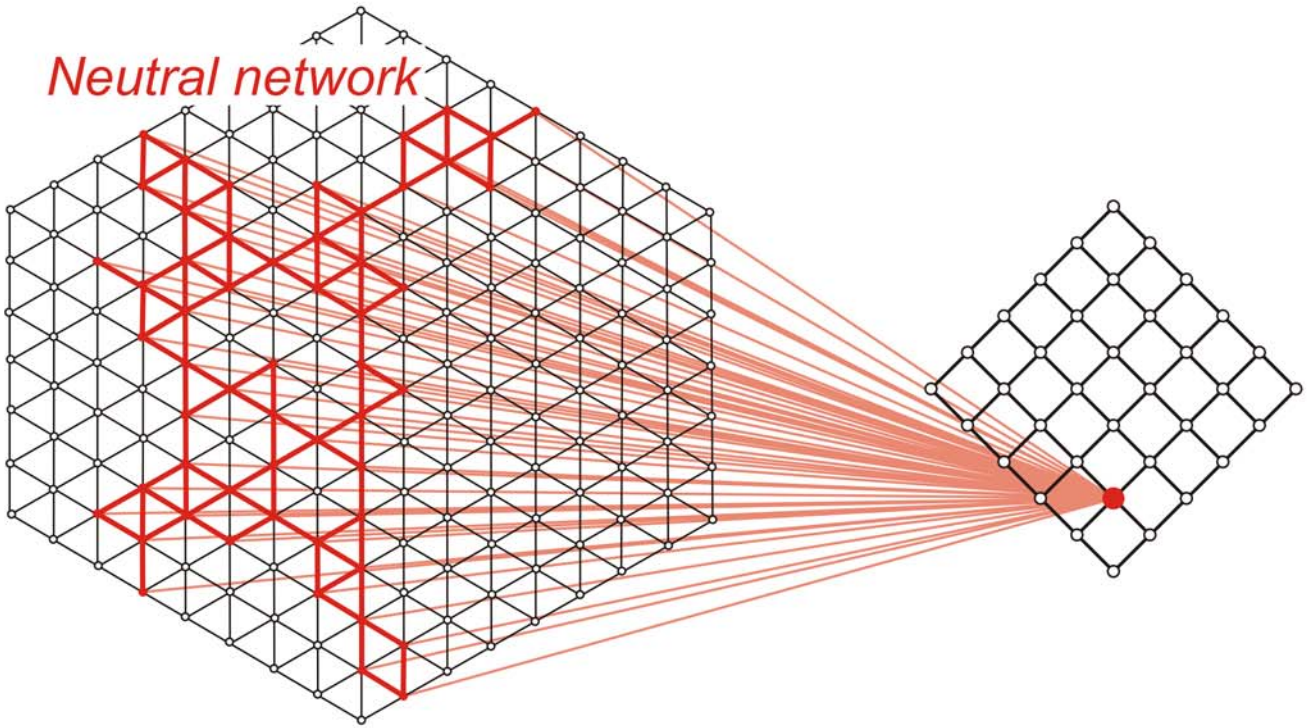
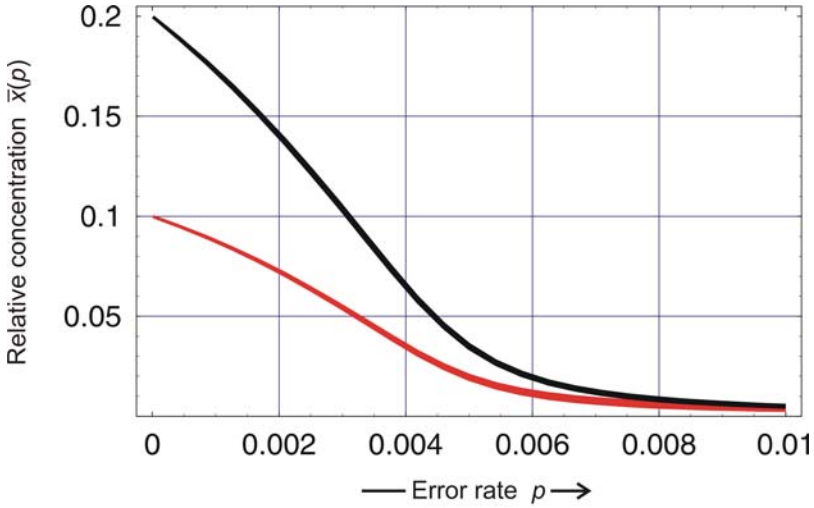
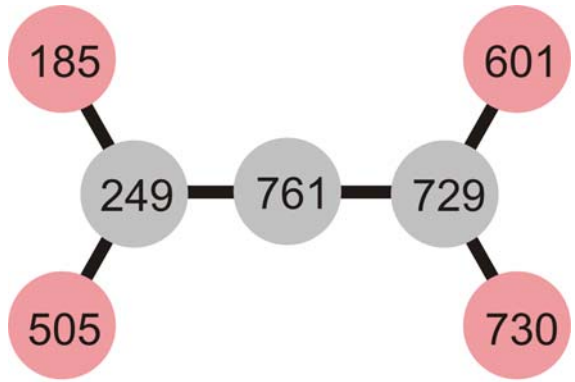

Neutral network
Sequence space
Structure space



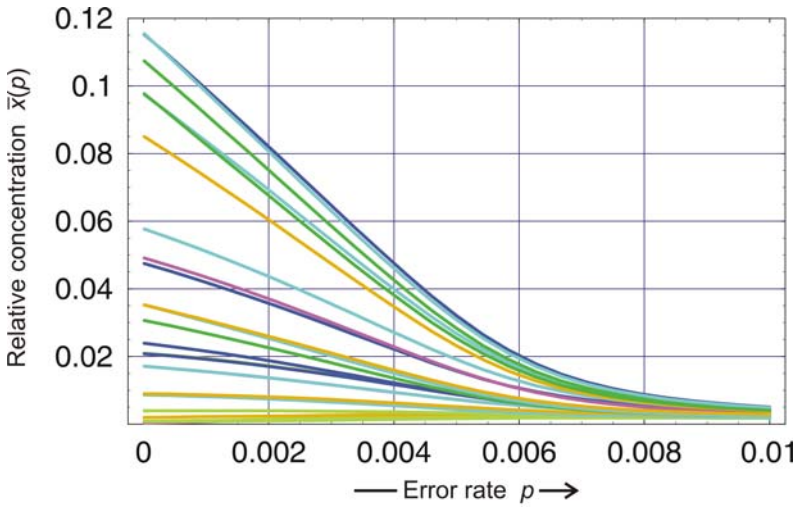


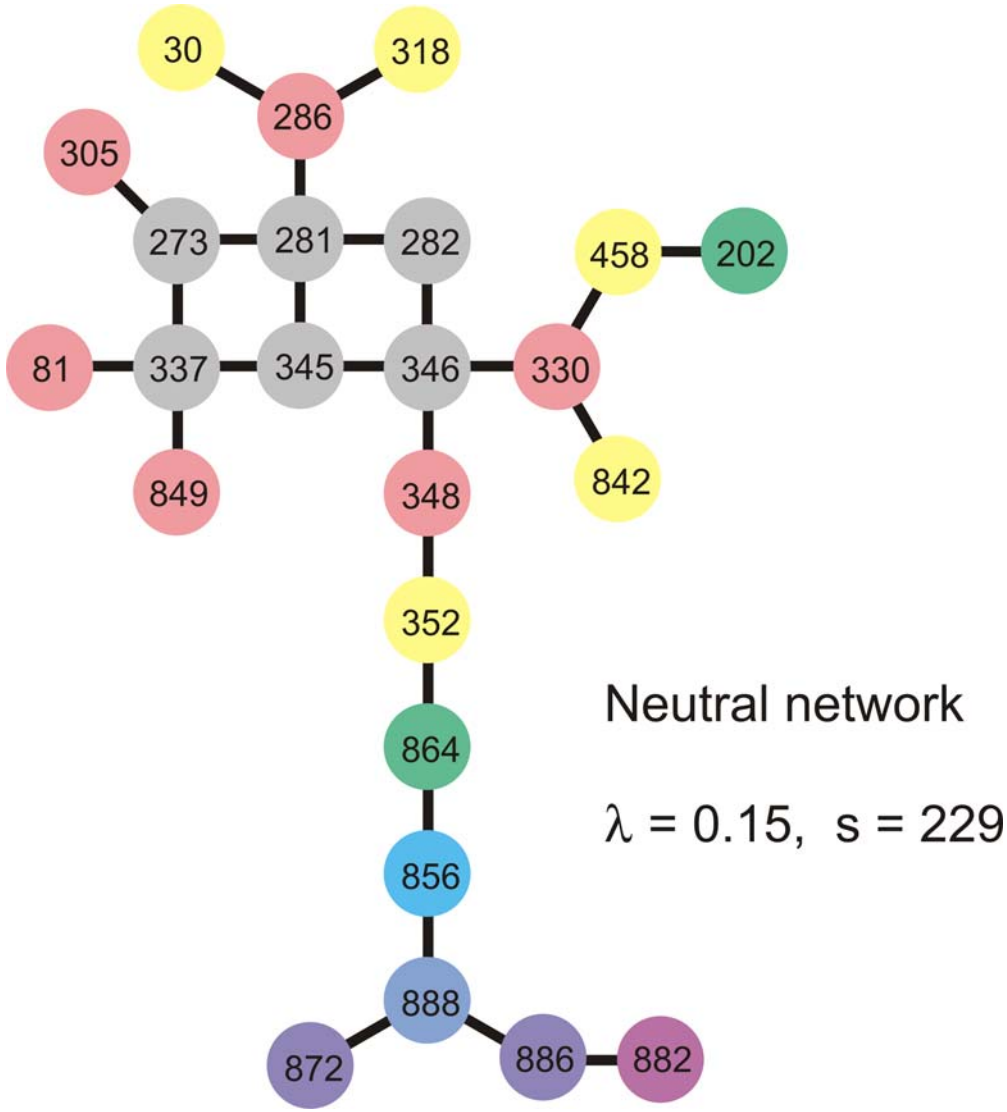
Neutral network

$\lambda = 0.10,\ s = 229$

N = 7

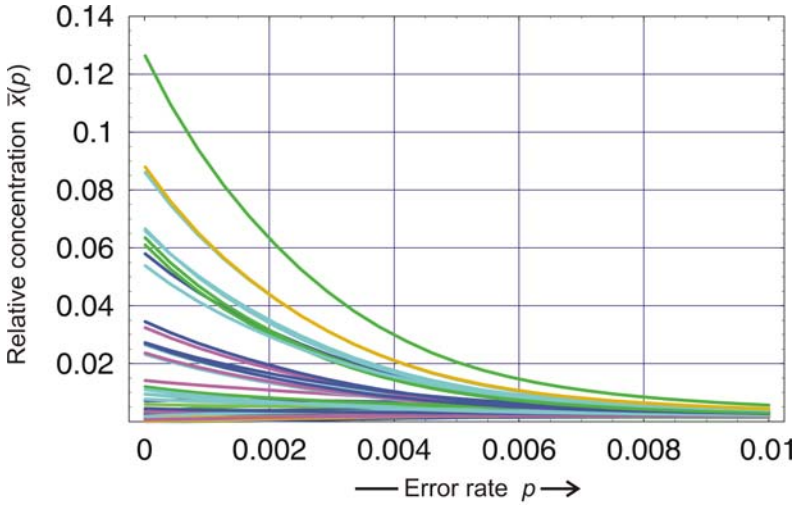
Neutral networks with
increasing λ

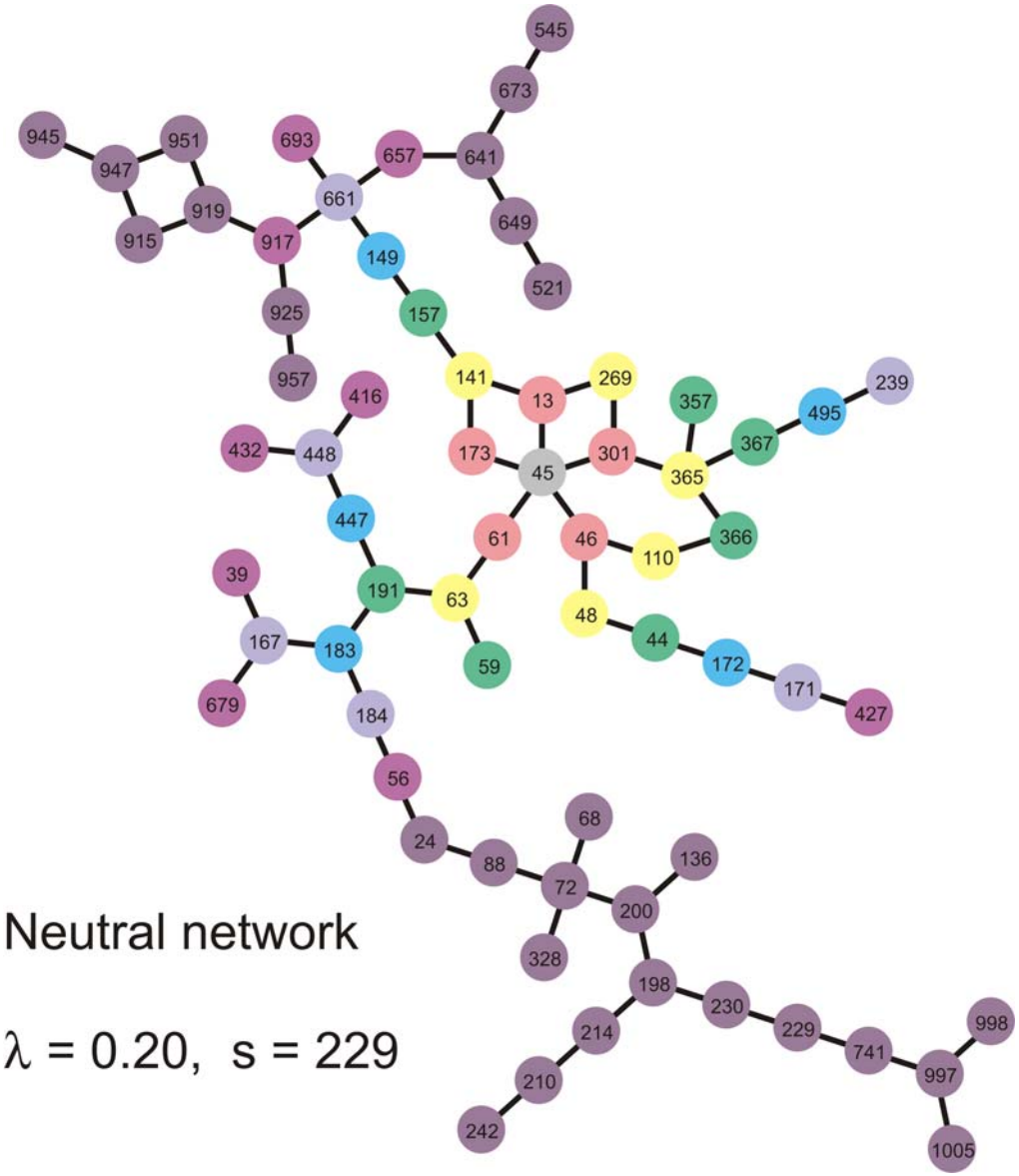




N = 24

Neutral networks with increasing λ



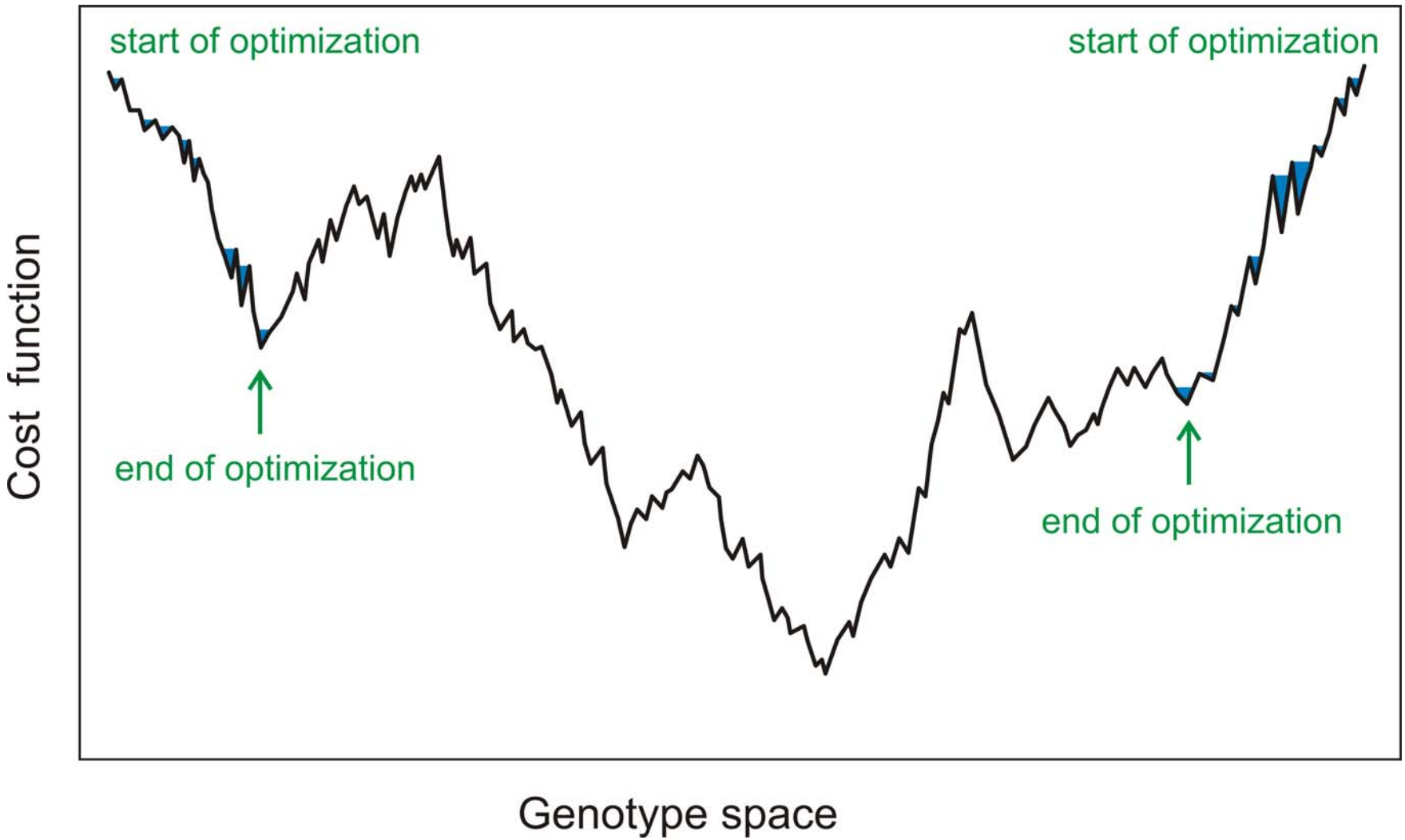


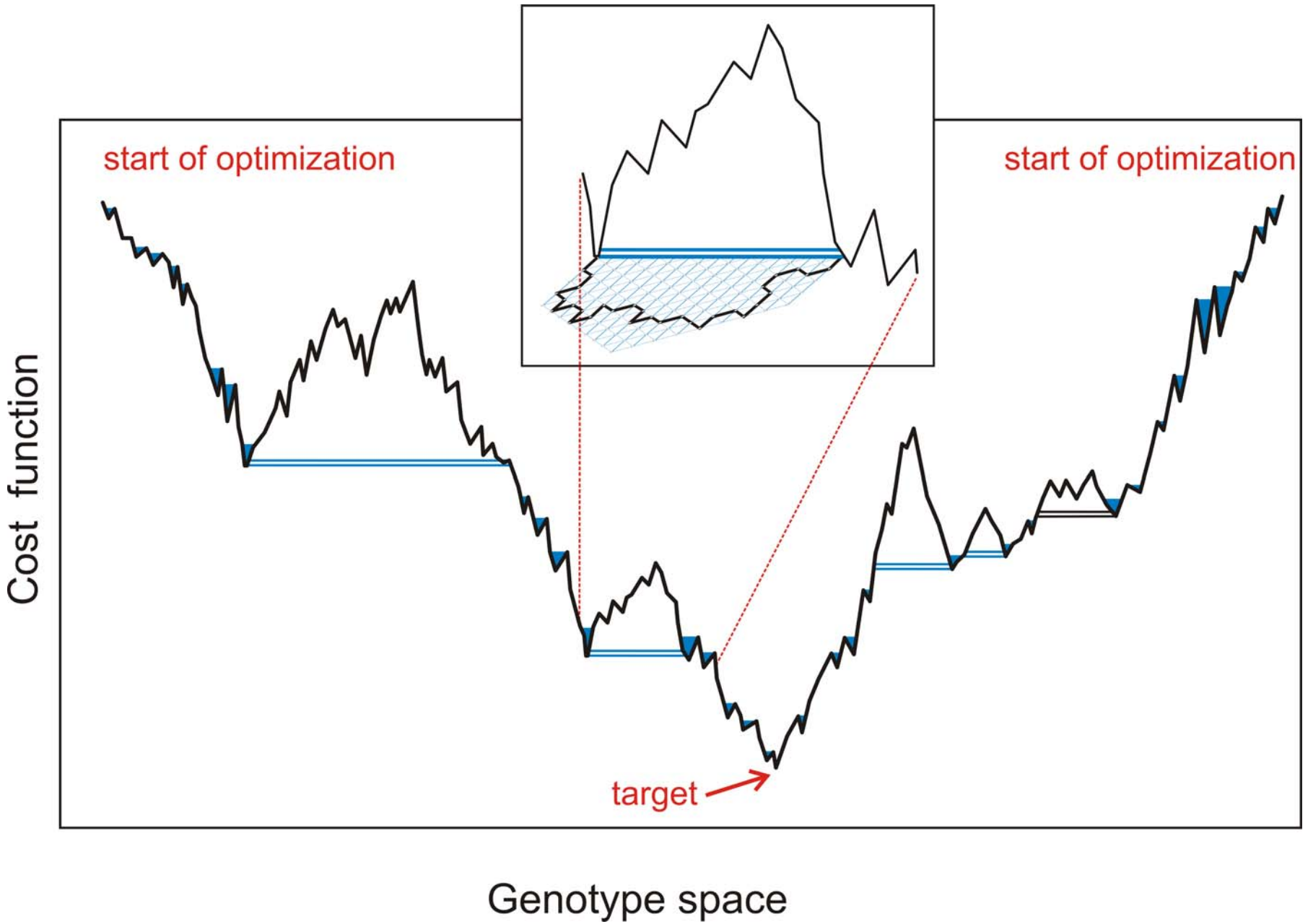
Neutral network

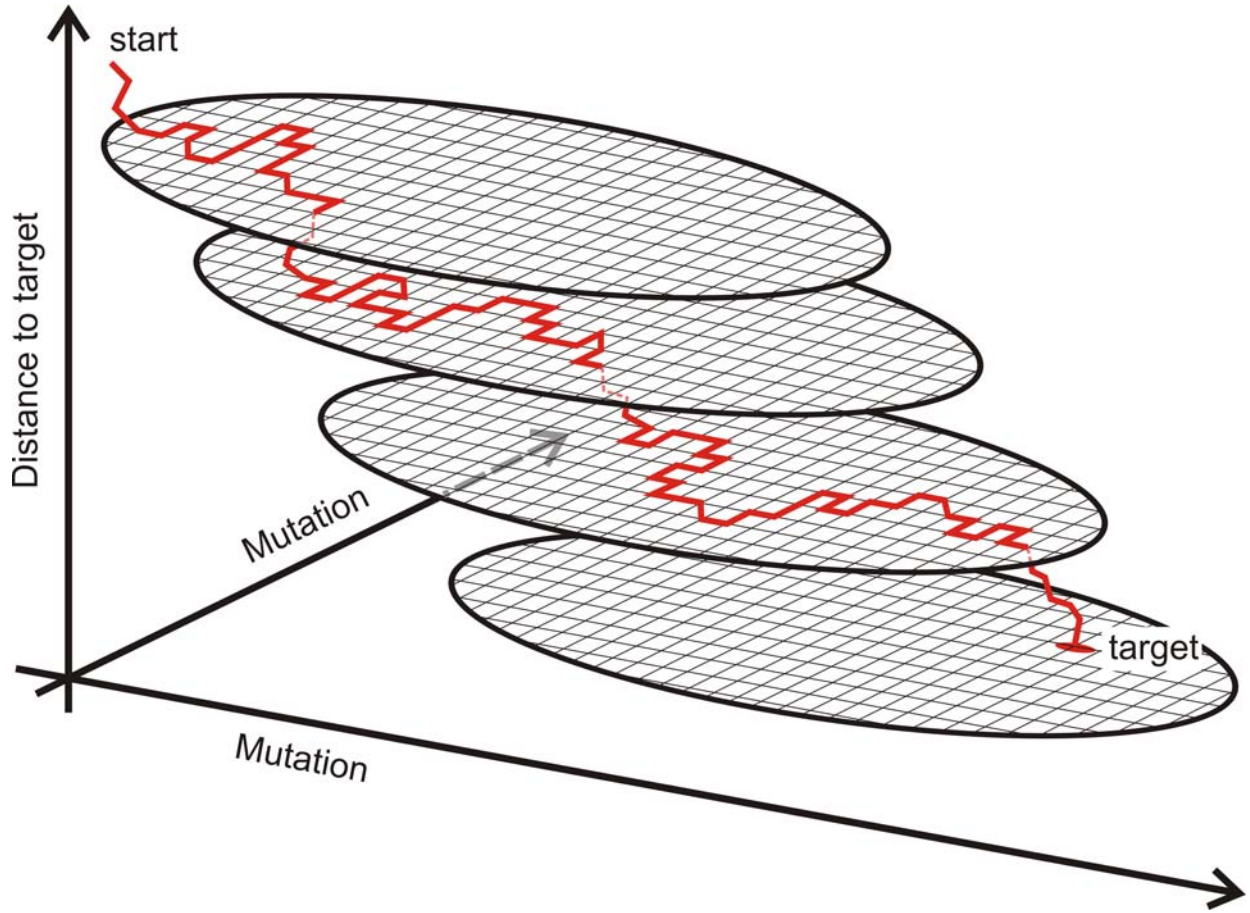
$\lambda = 0.20,\ s = 229$

N = 68

Neutral networks with increasing λ

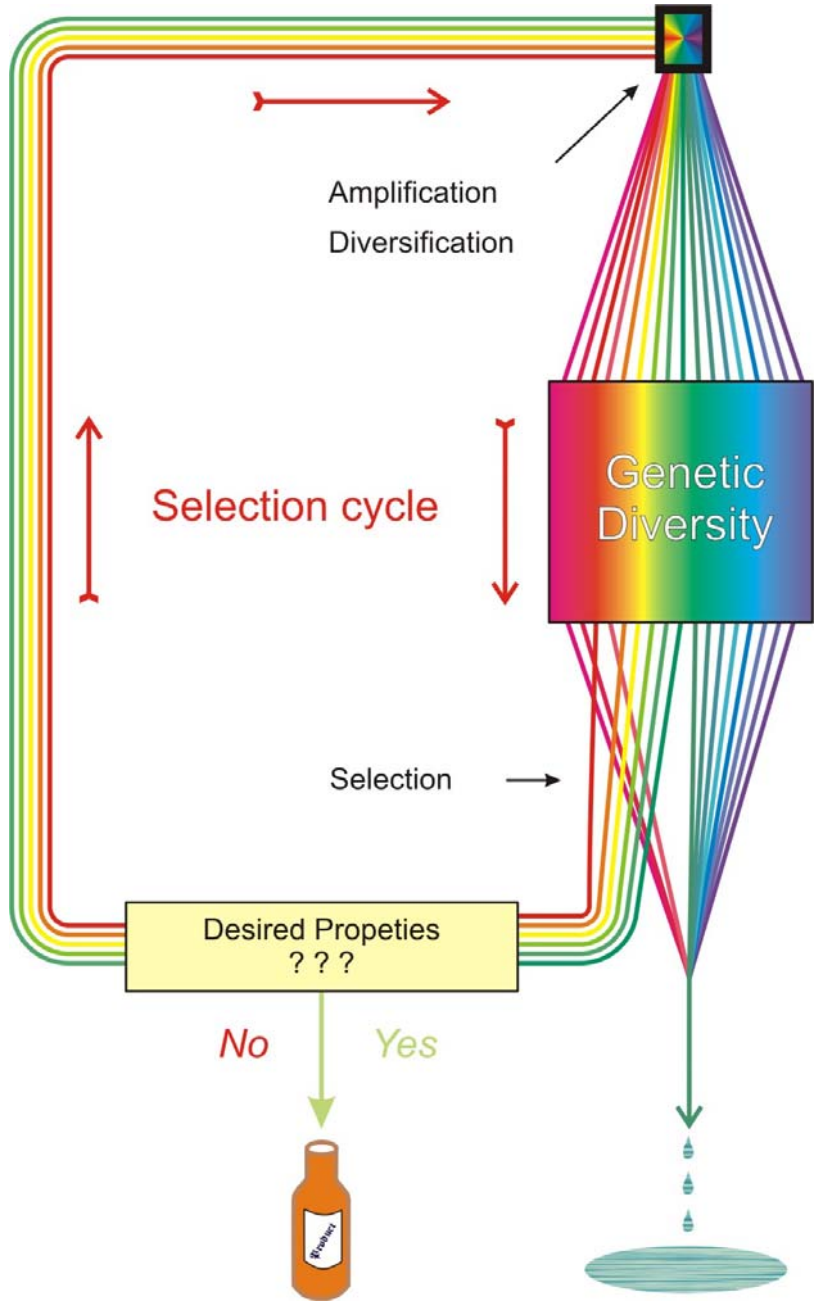

start of optimization
start of optimization
end of optimization
end of optimization
Cost function
Genotype space

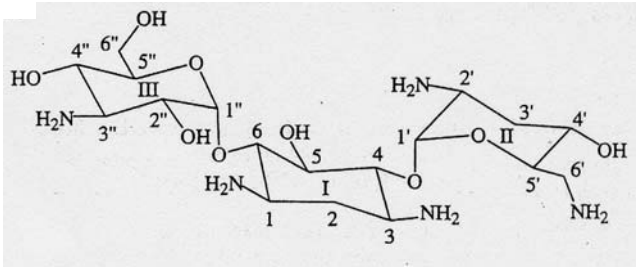

start of optimization
start of optimization
target
Cost function
Genotype space



A sketch of optimization on neutral networks

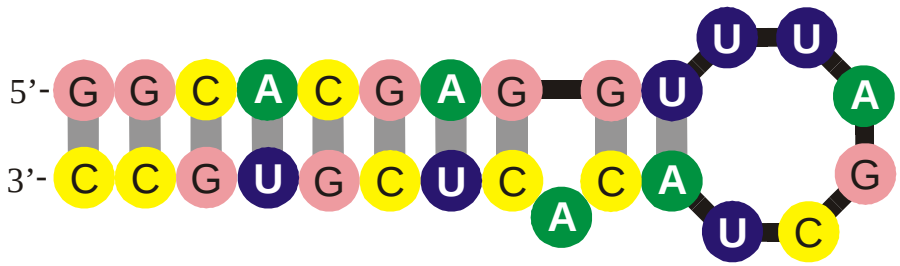
An example of selection of molecules with predefined properties in laboratory experiments





tobramycin

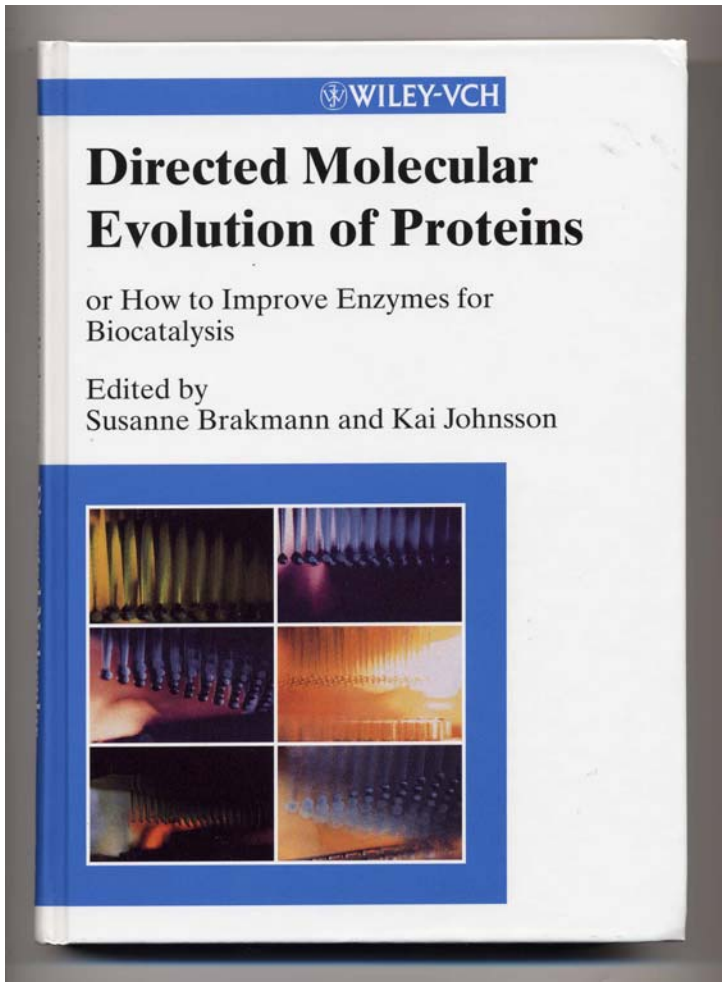
5’- G G C A C G A G G U U U A G C U A C A C U C G U G C C -3’

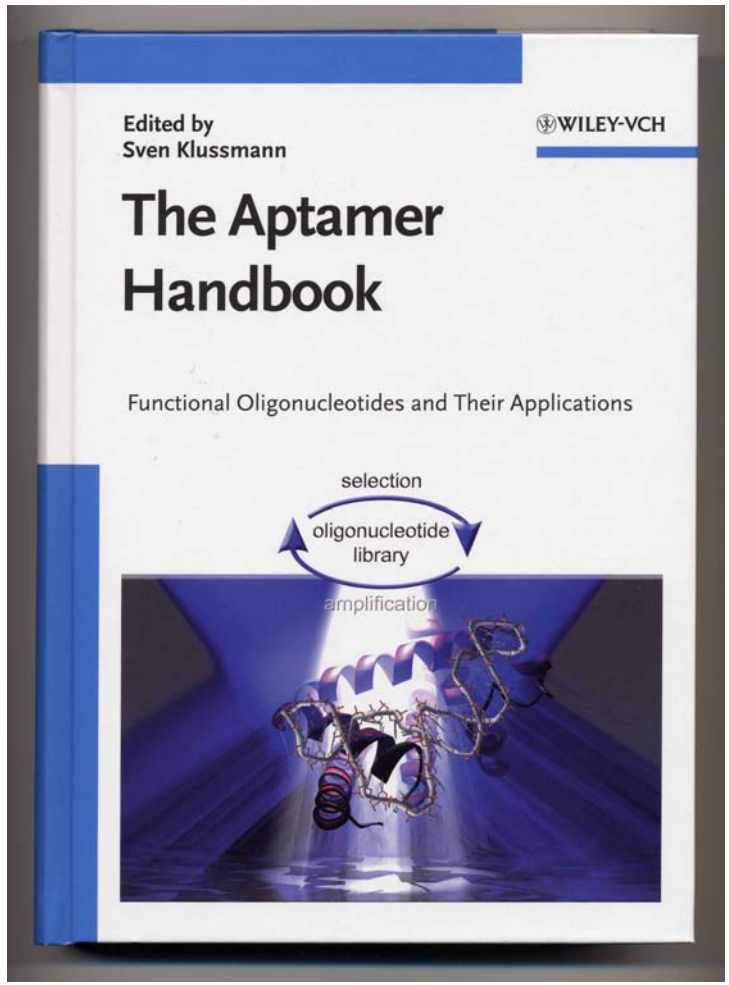


RNA aptamer

Secondary structure of the tobramycin binding RNA aptamer with K_D = 9 nM

L. Jiang, A. K. Suri, R. Fiala, D. J. Patel, ***Saccharide-RNA recognition in an aminoglycoside antibiotic-RNA aptamer complex.*** Chemistry & Biology **4**:35-50 (1997)





Application of molecular evolution to problems in biotechnology

Results from molecular evolution in laboratory experiments:

- Evolutionary optimization does not require cells and occurs in molecular systems too.

- *In vitro* evolution allows for production of molecules for predefined purposes and gave rise to a branch of biotechnology.

- Direct evidence that neutrality is a major factor for the success of evolution.

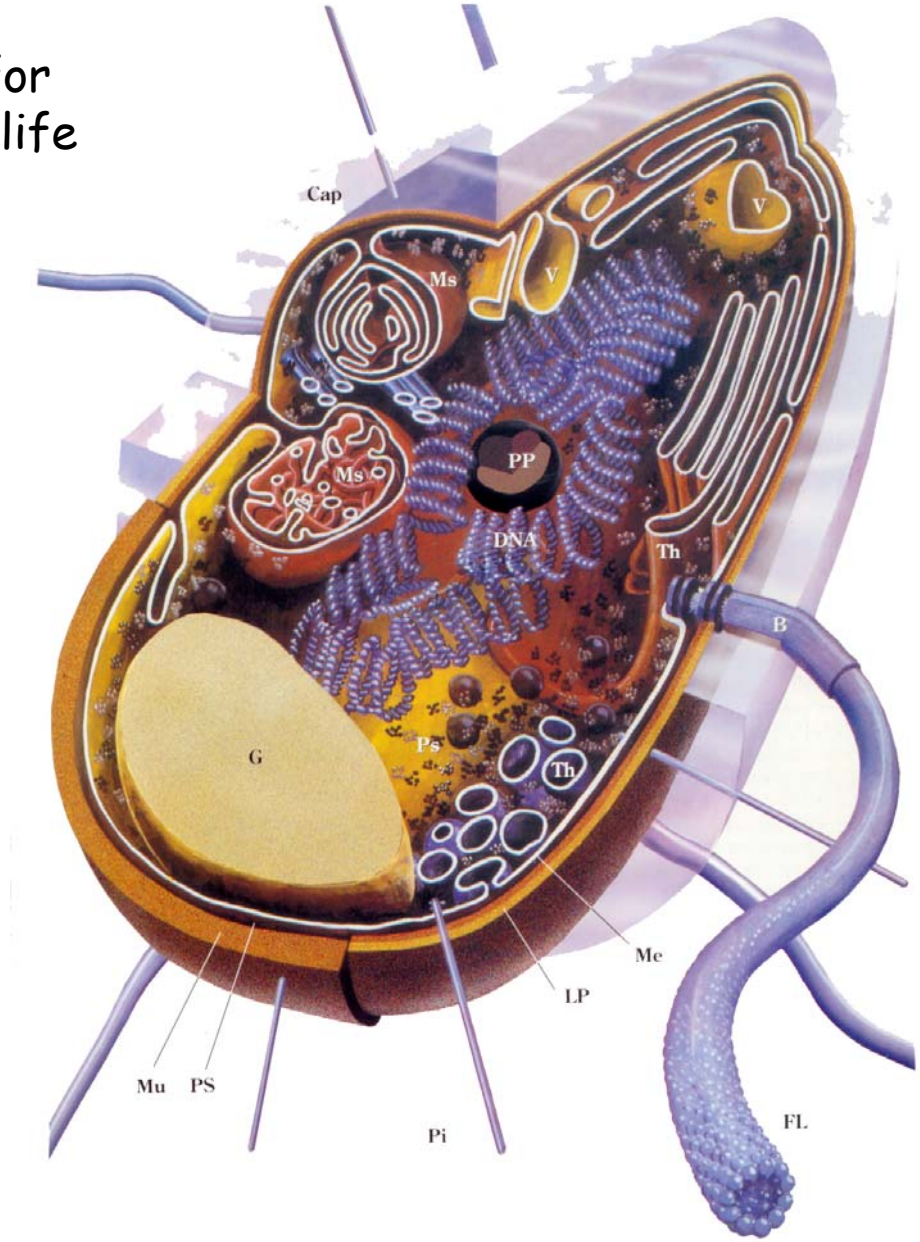
- Novel antiviral strategies were developed from known molecular mechanisms of virus evolution.

The bacterial cell as an example for the simplest form of autonomous life

Escherichia coli genome:

4 million nucleotides

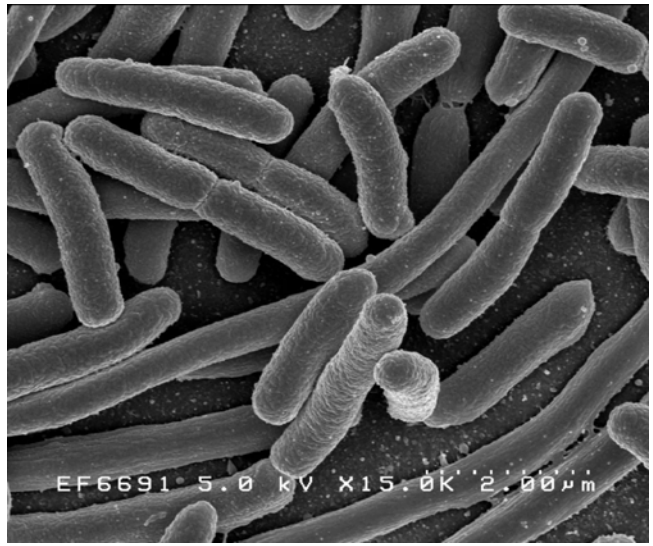
4460 genes



The structure of the bacterium *Escherichia coli*

E. coli:	Genome length	4×10^6 nucleotides
	Number of cell types	1
	Number of genes	4 460

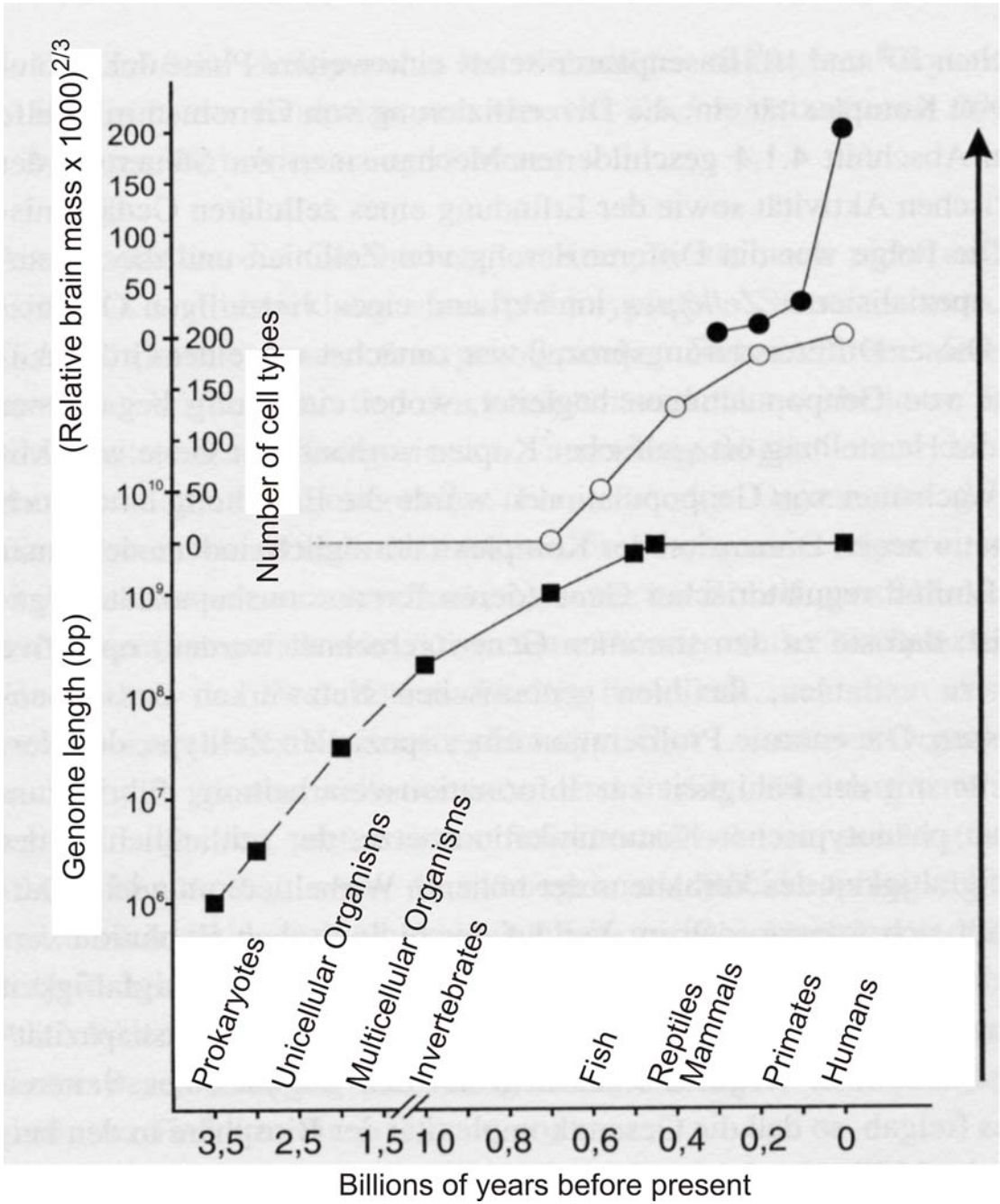
Four books, 300 pages each



Man:	Genome length	3×10^9 nucleotides
	Number of cell types	200
	Number of genes	≈ 30 000

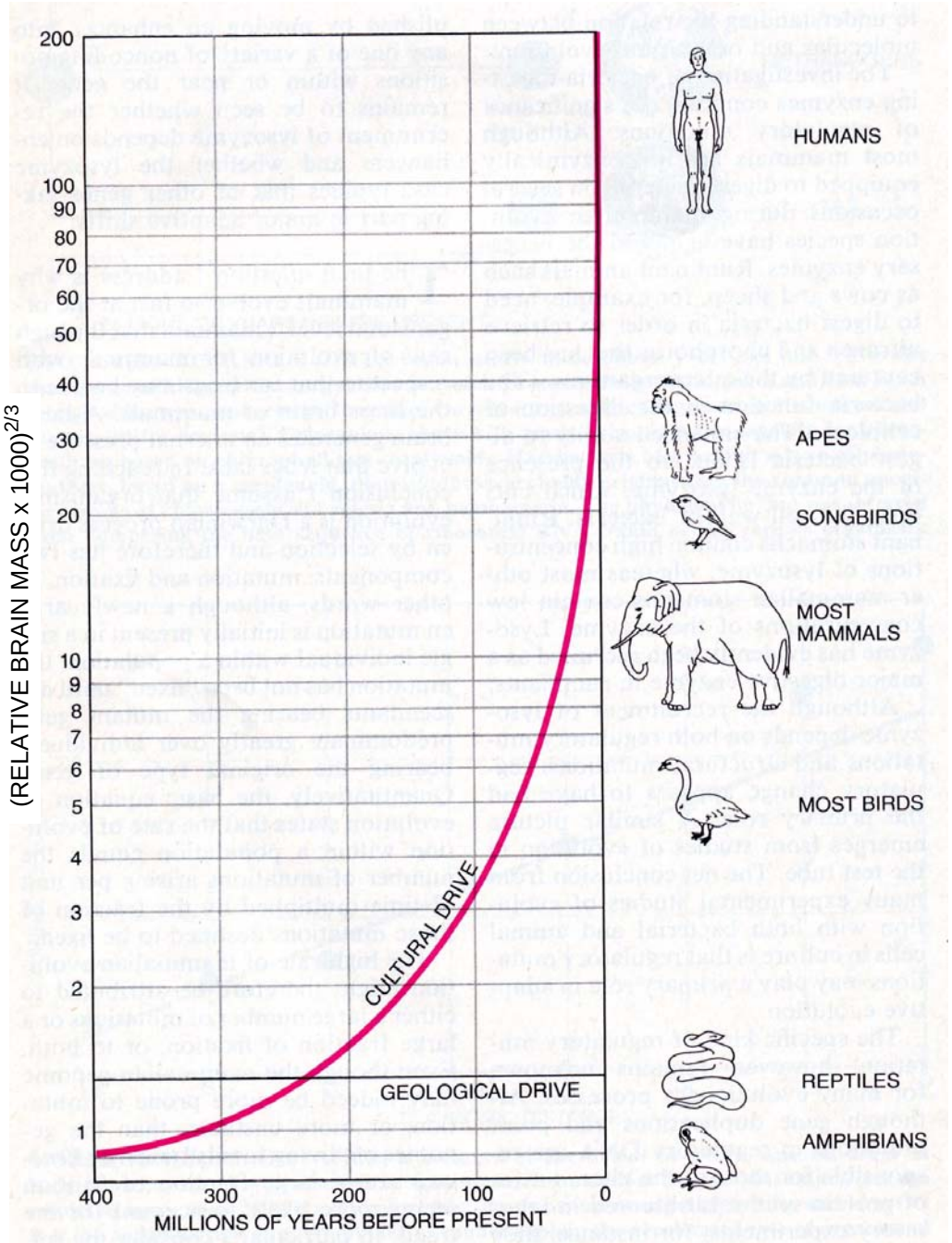
A library of 3000 volumes,
300 pages each

Complexity in biology



Wolfgang Wieser. 1998. ‚*Die Erfindung der Individualität*‘ oder ‚*Die zwei Gesichter der Evolution*‘. Spektrum Akademischer Verlag, Heidelberg 1998

BRITISH TIT



Alan C. Wilson.1985. The molecular basis of evolution.
Scientific American **253**(4):148-157.

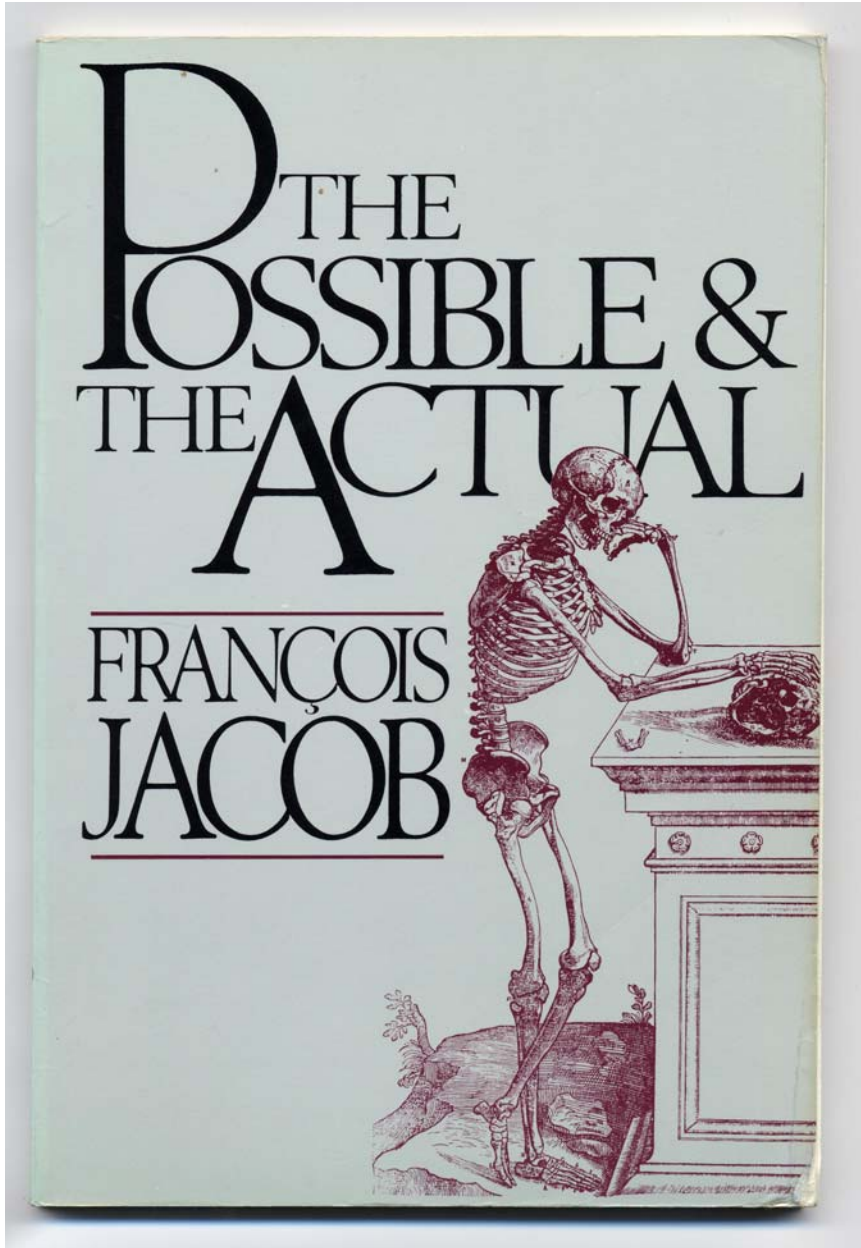
EVOLUTIONARY TINKERING

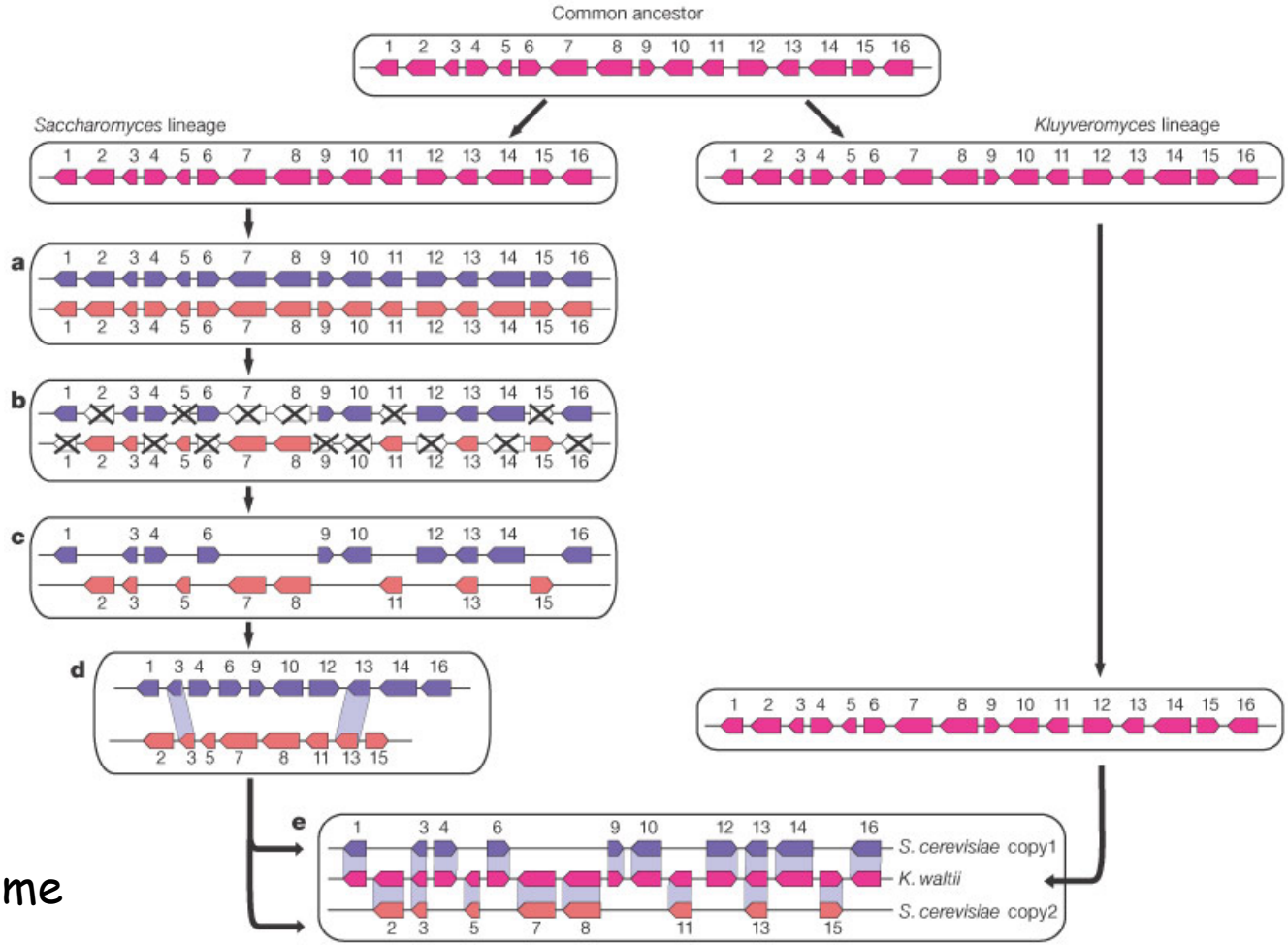
Blood . . . is the best possible thing to have coursing through one's veins.

—*Woody Allen*, Getting Even

Evolution does not design with the eyes of an engineer, evolution works like a tinkerer.

Francois Jacob, Pantheon Books, New York 1982





A model for the genome duplication in yeast 100 million years ago

Manolis Kellis, Bruce W. Birren, and Eric S. Lander. Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae*. *Nature* **428**: 617-624, 2004

The difficulty to define the notion of „gene".

Helen Pearson,
Nature **441**: 399-401, 2006

WHAT IS A GENE?

The idea of genes as beads on a DNA string is fast fading. Protein-coding sequences have no clear beginning or end and RNA is a key part of the information package, reports **Helen Pearson**.

'Gene' is not a typical four-letter word. It is not offensive. It is never bleeped out of TV shows. And where the meaning of most four-letter words is all too clear, that of gene is not. The more expert scientists become in molecular genetics, the less easy it is to be sure about what, if anything, a gene actually is.

Rick Young, a geneticist at the Whitehead Institute in Cambridge, Massachusetts, says that when he first started teaching as a young professor two decades ago, it took him about two hours to teach fresh-faced undergraduates what a gene was and the nuts and bolts of how it worked. Today, he and his colleagues need three months of lectures to convey the concept of the gene, and that's not because the students are any less bright. "It takes a whole semester to teach this stuff to talented graduates," Young says. "It used to be we could give a one-off definition and now it's much more complicated."

In classical genetics, a gene was an abstract concept — a unit of inheritance that ferried a characteristic from parent to child. As biochemistry came into its own, those characteristics were associated with enzymes or proteins, one for each gene. And with the advent of molecular biology, genes became real, physical things — sequences of DNA which when converted into strands of so-called messenger RNA could be used as the basis for building their associated protein piece by piece. The great coiled DNA molecules of the chromosomes were seen as long strings on which gene sequences sat like discrete beads.

This picture is still the working model for many scientists. But those at the forefront of genetic research see it as increasingly old-fashioned — a crude approximation that, at best, hides fascinating new complexities and, at worst, blinds its users to useful new paths of enquiry.

Information, it seems, is parceled out along chromosomes in a much more complex way than was originally supposed. RNA molecules are not just passive conduits through which the gene's message flows into the world but active regulators of cellular processes. In some cases, RNA may even pass information across generations — normally the sole preserve of DNA.

An eye-opening study last year raised the possibility that plants sometimes rewrite their DNA on the basis of RNA messages inherited from generations past[1]. A study on page 469 of this issue suggests that a comparable phenomenon might occur in mice, and by implication in other mammals[2]. If this type of phenomenon is indeed widespread, it "would have huge implications," says evolutionary geneticist Laurence Hurst at the University of Bath, UK.

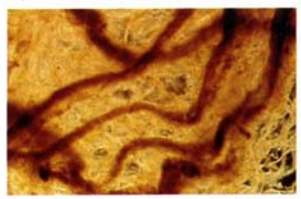
"All of that information seriously challenges our conventional definition of a gene," says molecular biologist Bing Ren at the University of California, San Diego. And the information challenge is about to get even tougher. Later this year, a glut of data will be released from the international Encyclopedia of DNA Elements (ENCODE) project. The pilot phase of ENCODE involves scrutinizing roughly 1% of the human genome in unprecedented detail; the aim is to find all the sequences that serve a useful purpose and explain what that purpose is. "When we started the ENCODE project I had a different view of what a gene was," says contributing researcher Roderic Guigo at the Center for Genomic Regulation in Barcelona. "The degree of complexity we've seen was not anticipated."

"We've come to the realization that the genome is full of overlapping transcripts."
— Phillip Kapranov

Under fire

The first of the complexities to challenge molecular biology's paradigm of a single DNA sequence encoding a single protein was alternative splicing, discovered in viruses in 1977 (see 'Hard to track', overleaf). Most of the DNA sequences describing proteins in humans have a modular arrangement in which exons, which carry the instructions for making proteins, are interspersed with non-coding introns. In alternative splicing, the cell snips out introns and sews together the exons in various different orders, creating messages that can code for different proteins. Over the years geneticists have also documented overlapping genes, genes within genes and countless other weird arrangements (see 'Muddling over genes', overleaf).

Alternative splicing, however, did not in itself require a drastic reappraisal of the notion of a gene; it just showed that some DNA sequences could describe more than one protein. Today's assault on the gene concept is more far reaching, fuelled largely by studies that show the previously unimagined scope of RNA.



Spools of DNA (above) still harbour surprises, with one protein-coding gene often overlapping the next.

P. PLAILLY/SPL

The one gene, one protein idea is coming under particular assault from researchers who are comprehensively extracting and analysing the RNA messages, or transcripts, manufactured by genomes, including the human and mouse genome. Researchers led by Thomas Gingeras at the company Affymetrix in Santa Clara, California, for example, recently studied all the transcripts from ten chromosomes across eight human cell lines and worked out precisely where on the chromosomes each of the transcripts came from[3].

The picture these studies paint is one of mind-boggling complexity. Instead of discrete genes dutifully mass-producing identical RNA transcripts, a teeming mass of transcription converts many segments of the genome into multiple RNA ribbons of differing lengths. These ribbons can be generated from both strands of DNA, rather than from just one as was conventionally thought. Some of these transcripts come from regions of DNA previously identified as holding protein-coding genes. But many do not. "It's somewhat revolutionary," says Gingeras's colleague Phillip Kapranov. "We've come to the realization that the genome is full of overlapping transcripts."

Other studies, one by Guigo's team[4], and one by geneticist Rotem Sorek[5], now at Tel Aviv University, Israel, and his colleagues, have hinted at the reasons behind the mass of transcription. The two teams investigated occasional reports that transcription can start at a DNA sequence associated with one protein and run straight through into the gene for a completely different protein, producing a fused transcript. By delving into databases of human RNA transcripts, Guigo's team estimate that 4–5% of the DNA in regions conventionally recognized as genes is transcribed in this way. Producing fused transcripts could be one way for a cell to generate a greater variety of proteins from a limited number of exons, the researchers say.

Many scientists are now starting to think that the descriptions of proteins encoded in DNA know no borders — that each sequence reaches into the next and beyond. This idea will be one of the central points to emerge from the ENCODE project when its results are published later this year.

Kapranov and others say that they have documented many examples of transcripts in which protein-coding exons from one part of the genome combine with exons from another

ENCODE stands for
ENCyclopedia Of DNA Elements.

ENCODE Project Consortium.
Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project.
Nature **447**:799-816, 2007

Fast and frugal heuristics use simple rules for

- guiding search for information,
- stopping search, and
- decision making.

E. Brandstätter, G. Gigerenzer, R. Herwig. 2006. The priority heuristic: Making choices without trade offs. *Psychological Review* 113:409-432.

Web-Page for further information:

http://www.tbi.univie.ac.at/~pks