# Evolution on „Realistic“ Landscapes

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Web-Page for further information:

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

1. History of „fitness landscape“
2. Molecular biology of replication
3. Simple landscapes
4. Landscapes revisited
5. „Realistic“ landscapes
6. Neutrality in evolution
7. Perspectives

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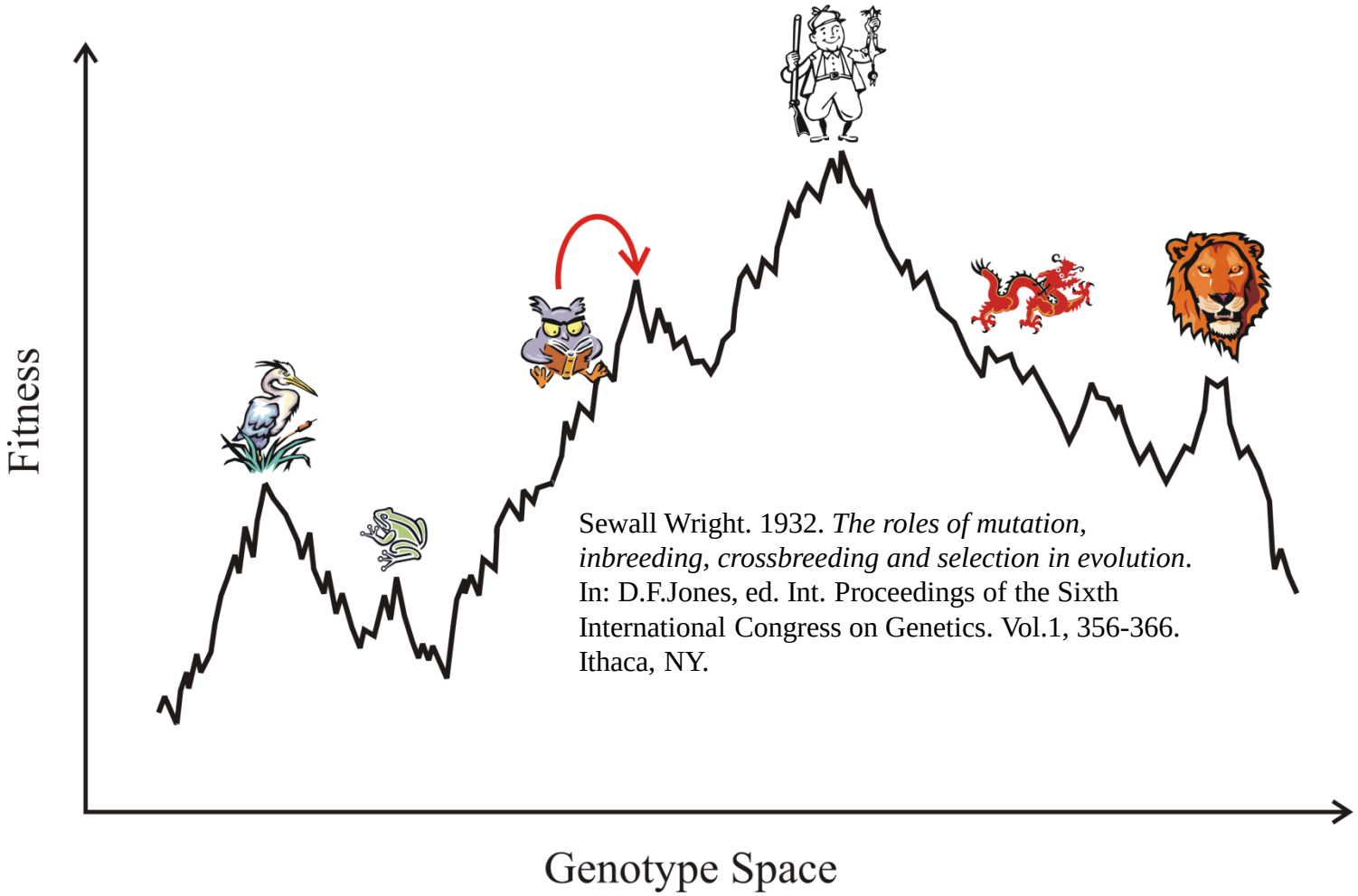



Sewall Wrights fitness landscape as metaphor for Darwinian evolution

Sewall Wright, 1889 - 1988

+ …….. wild type
***a*** ..... alternative allele on locus A
:
:
:
***abcde*** … alternative alleles on all five loci

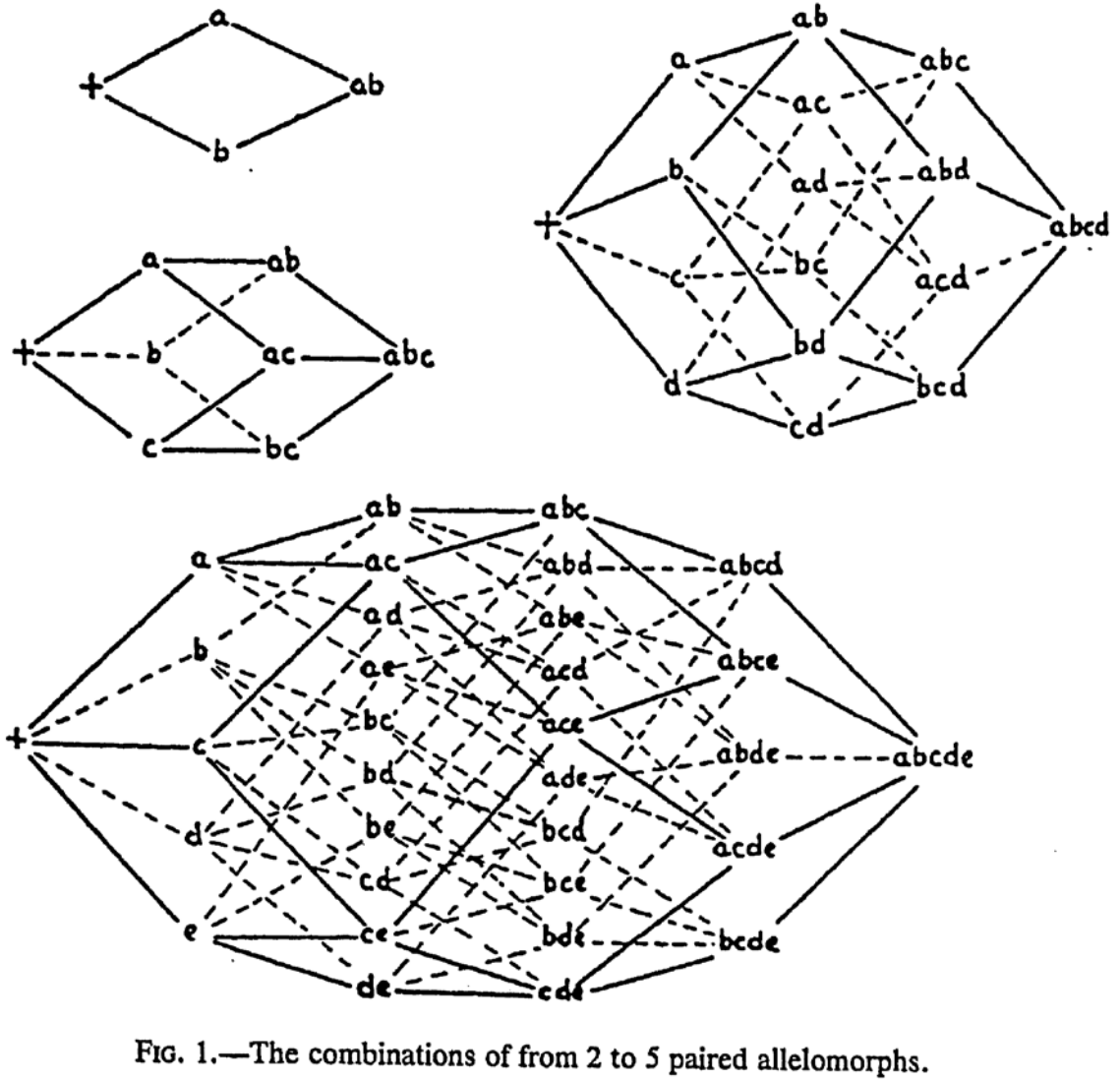
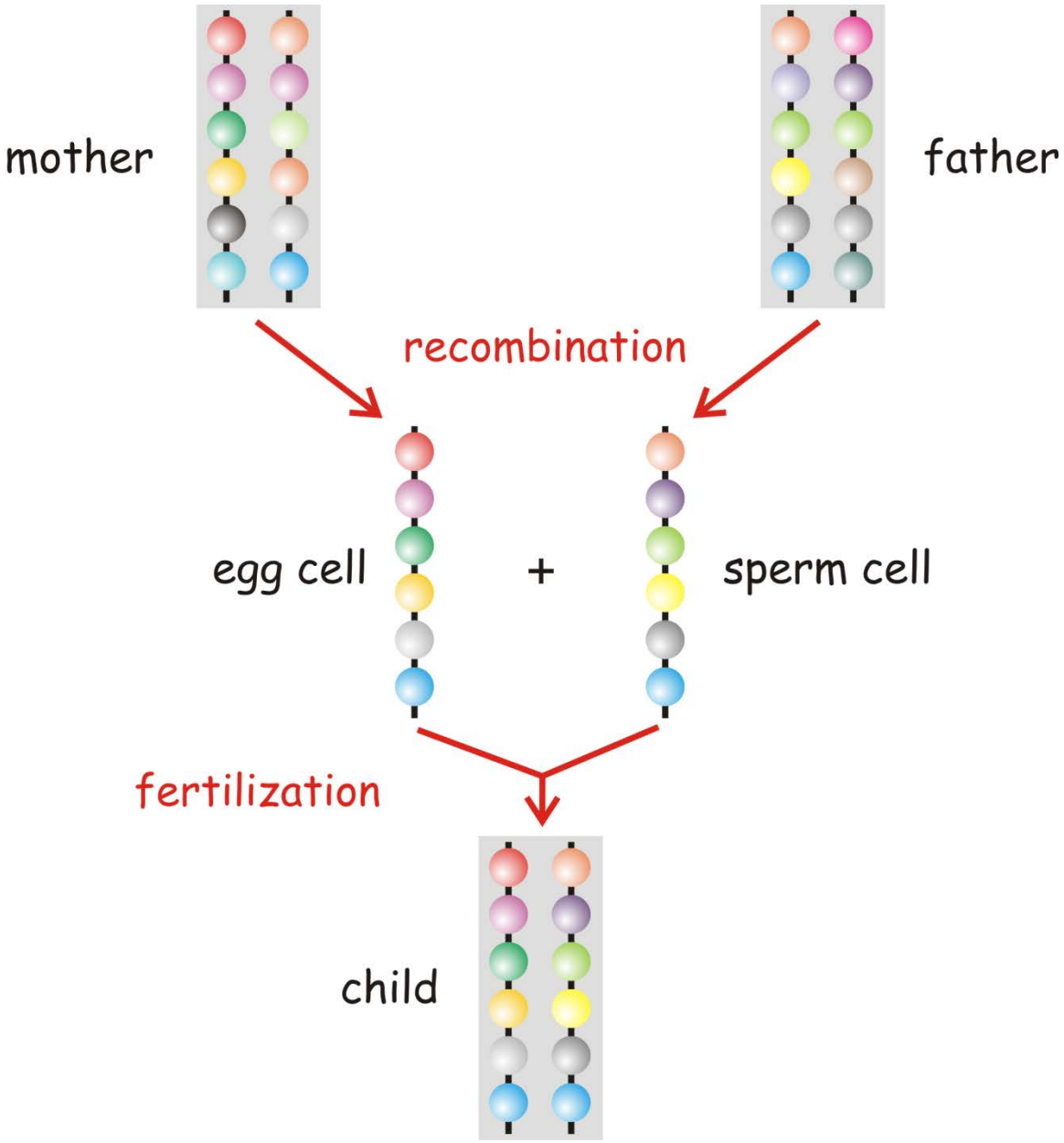



FIG. 1.—The combinations of from 2 to 5 paired allelomorphs.

The multiplicity of gene replacements with two alleles on each locus

Sewall Wright. 1988. Surfaces of selective value revisited. American Naturalist 131:115-123

Gregor Mendel
1822 - 1844




# Recombination in Mendelian genetics

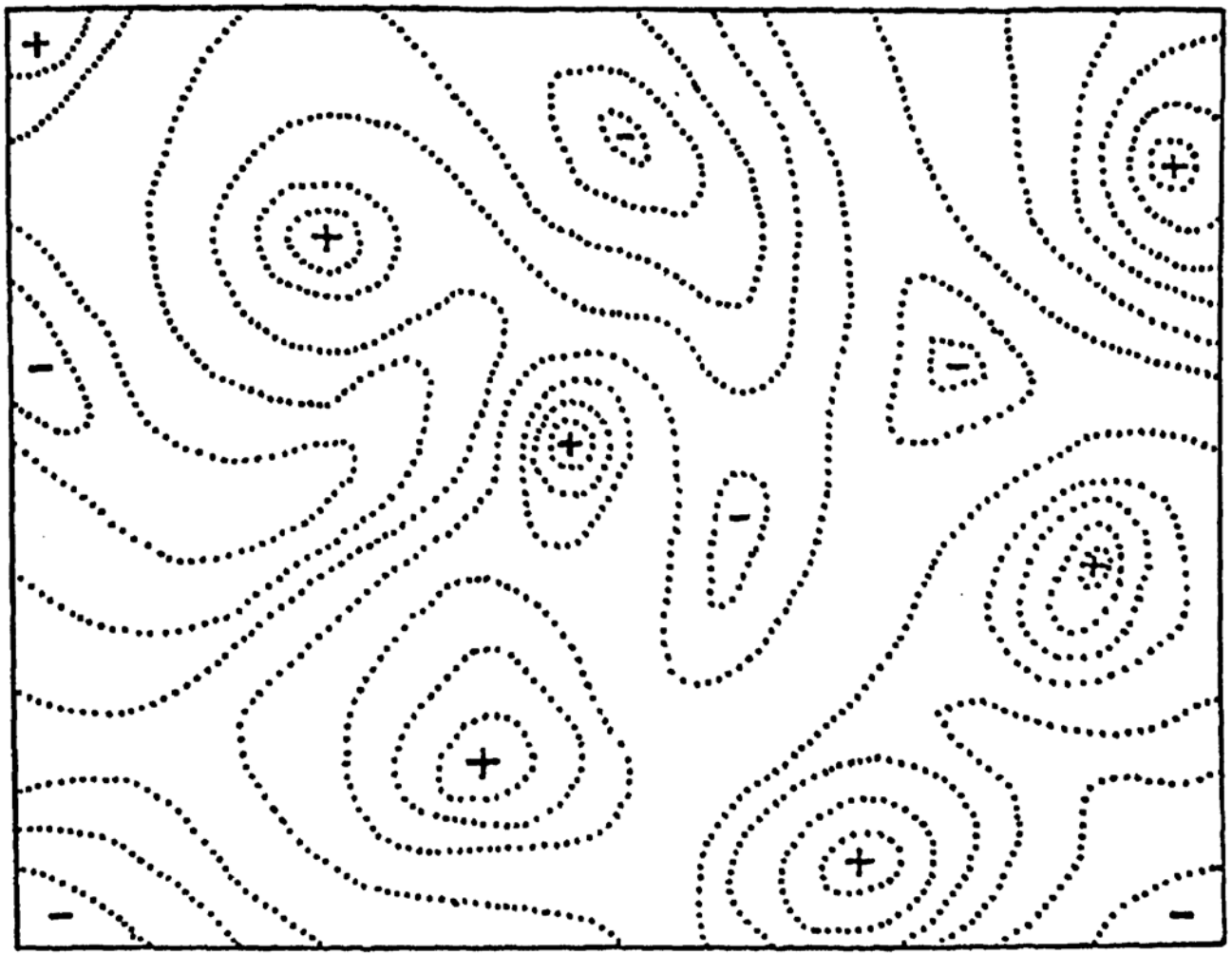
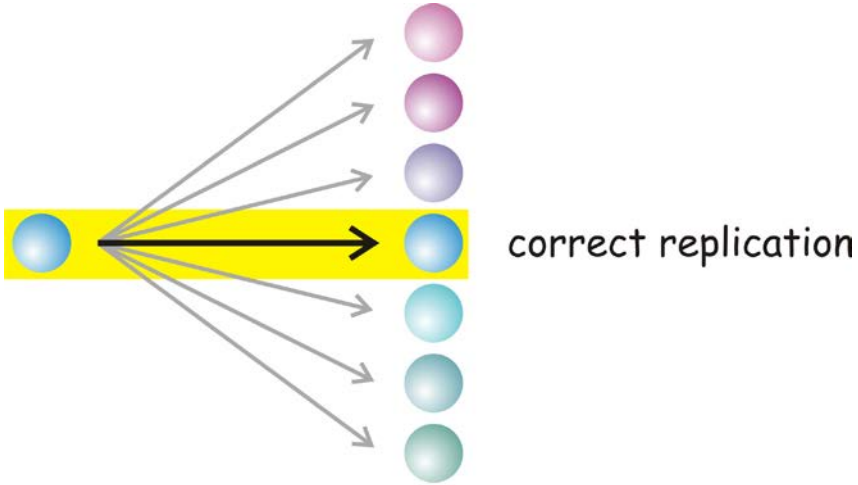


FIG. 2.—Diagrammatic representation of the field of gene combinations in two dimensions instead of many thousands. Dotted lines represent contours with respect to adaptiveness.

Evolution is hill climbing of populations or subpopulations

Sewall Wright. 1988. Surfaces of selective value revisited. American Naturalist 131:115-123

Hermann J. Muller
1890 - 1967




Thomas H. Morgan
1866 - 1945

| organism | mutation rate per genome | reproduction event |
|---|---|---|
| RNA virus | 1 | replication |
| retroviruses | 0.1 | replication |
| bacteria | 0.003 | replication |
| eukaryotes | 0.003 | cell division |
| eukaryotes | 0.01 – 0.1 | sexual reproduction |

John W. Drake, Brian Charlesworth, Deborah Charlesworth and James F. Crow. 1998. Rates of spontaneous mutation. *Genetics* 148:1667-1686.

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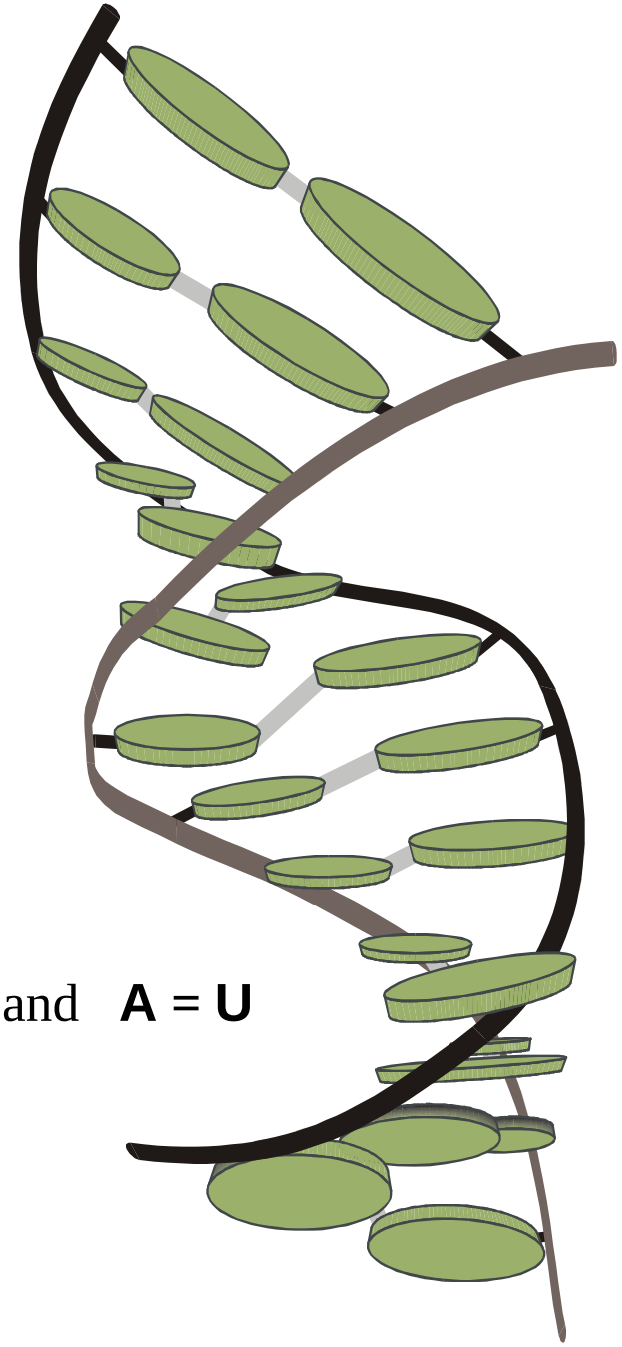
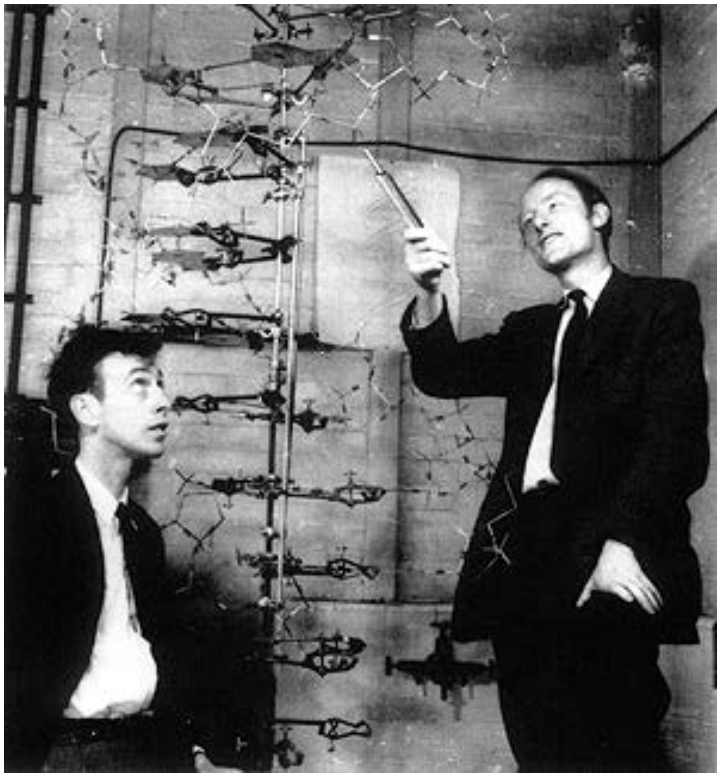
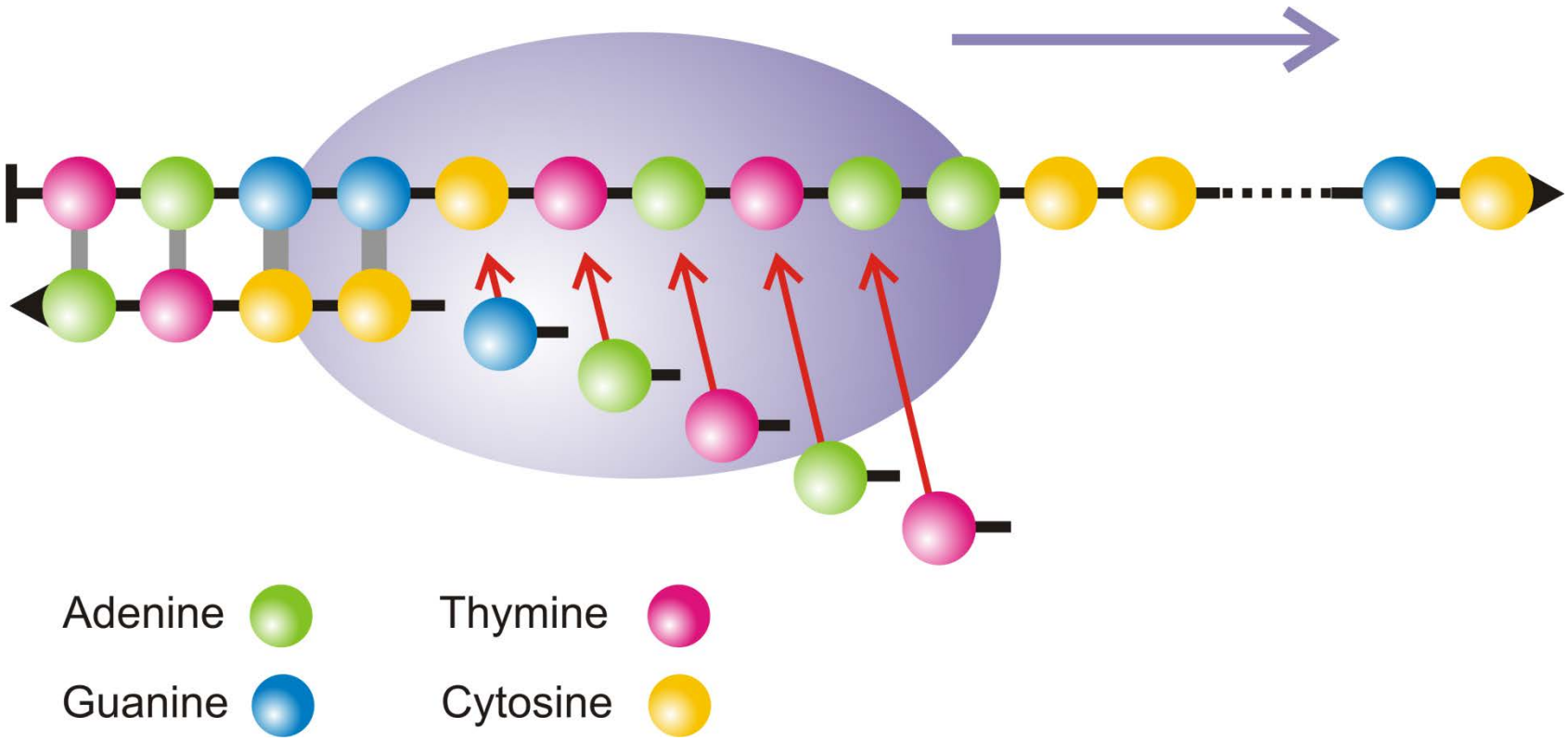


**G ≡ C** and **A = U**



James D. Watson, 1928- , and Francis Crick, 1916-2004,
Nobel Prize 1962

The three-dimensional structure of a short double helical stack of B-DNA




Accuracy of replication: $Q = q_1 \cdot q_2 \cdot q_3 \cdot q_4 \cdot \ldots$

The logics of DNA (or RNA) replication

DOI: 10.1002/anie.200701369

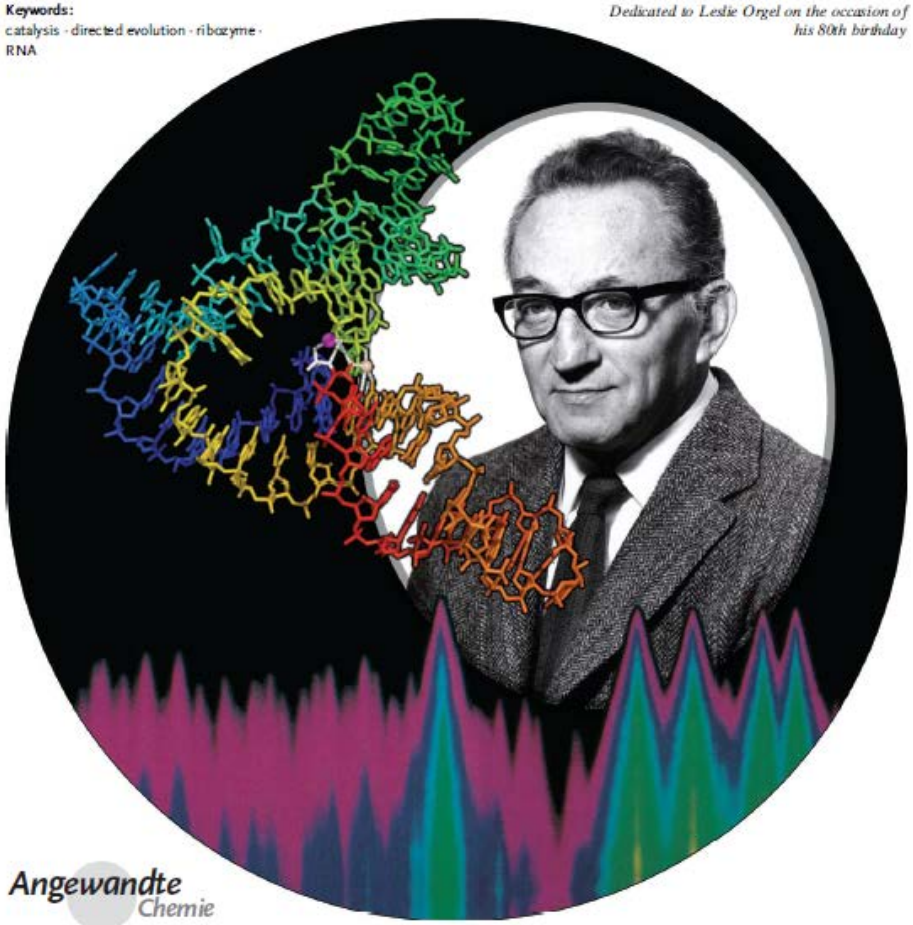
Molecular Evolution

# Forty Years of In Vitro Evolution**

*Gerald F. Joyce**

**Keywords:**
catalysis · directed evolution · ribozyme · RNA

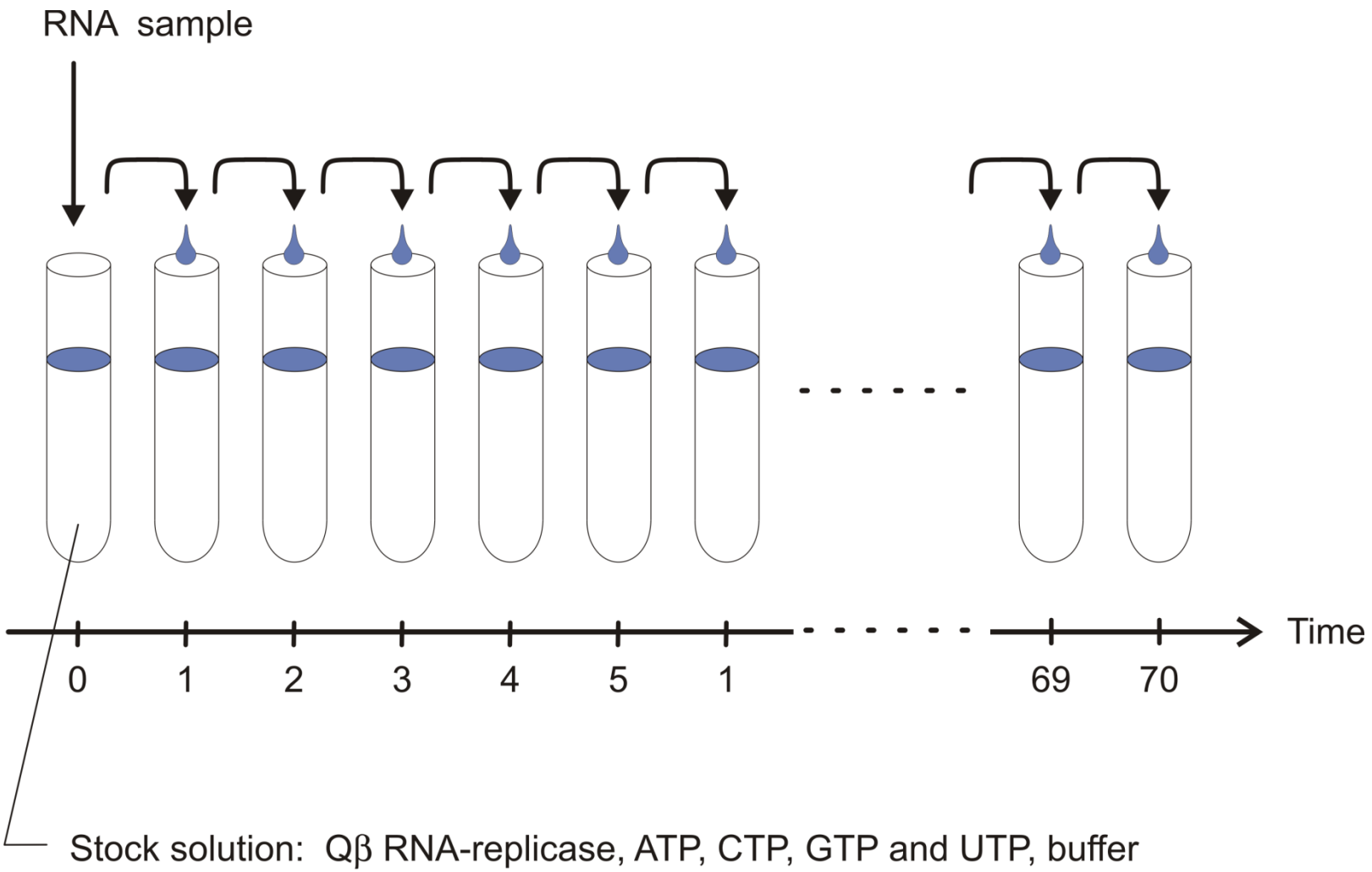
*Dedicated to Leslie Orgel on the occasion of his 80th birthday*



Sol Spiegelman,
1914 - 1983

Evolution in the test tube:

G.F. Joyce, *Angew.Chem.Int.Ed.* **46** (2007), 6420-6436


RNA sample
Time
0
1
2
3
4
5
1
69
70
Stock solution: Qβ RNA-replicase, ATP, CTP, GTP and UTP, buffer

Reproduction of the original figure of the serial transfer experiment with Qβ RNA

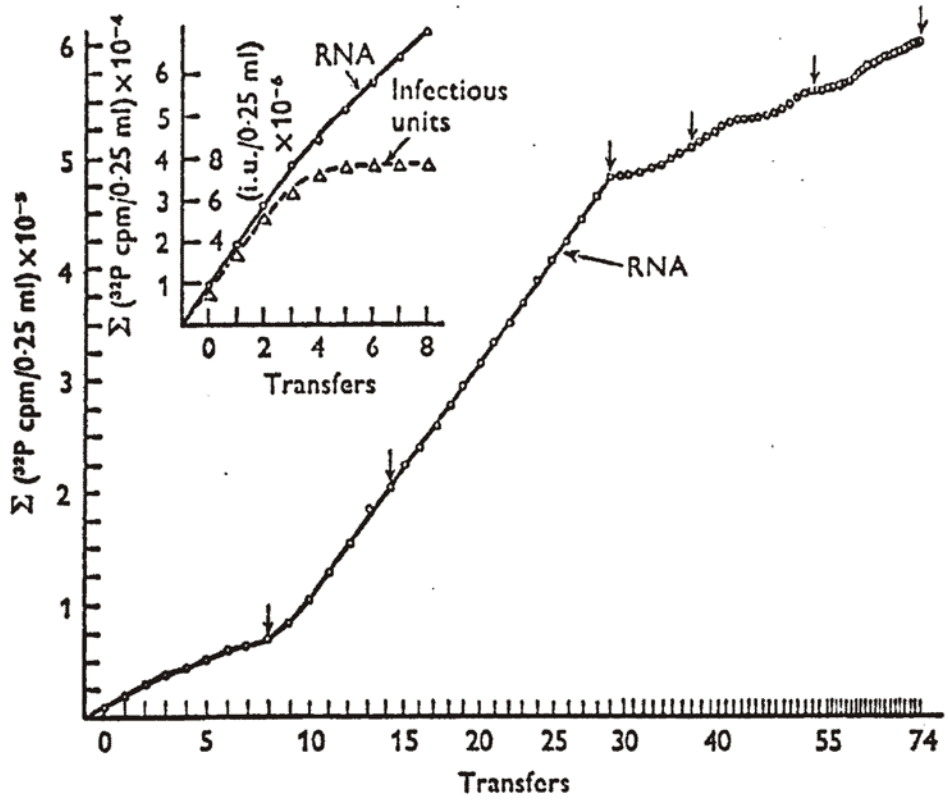


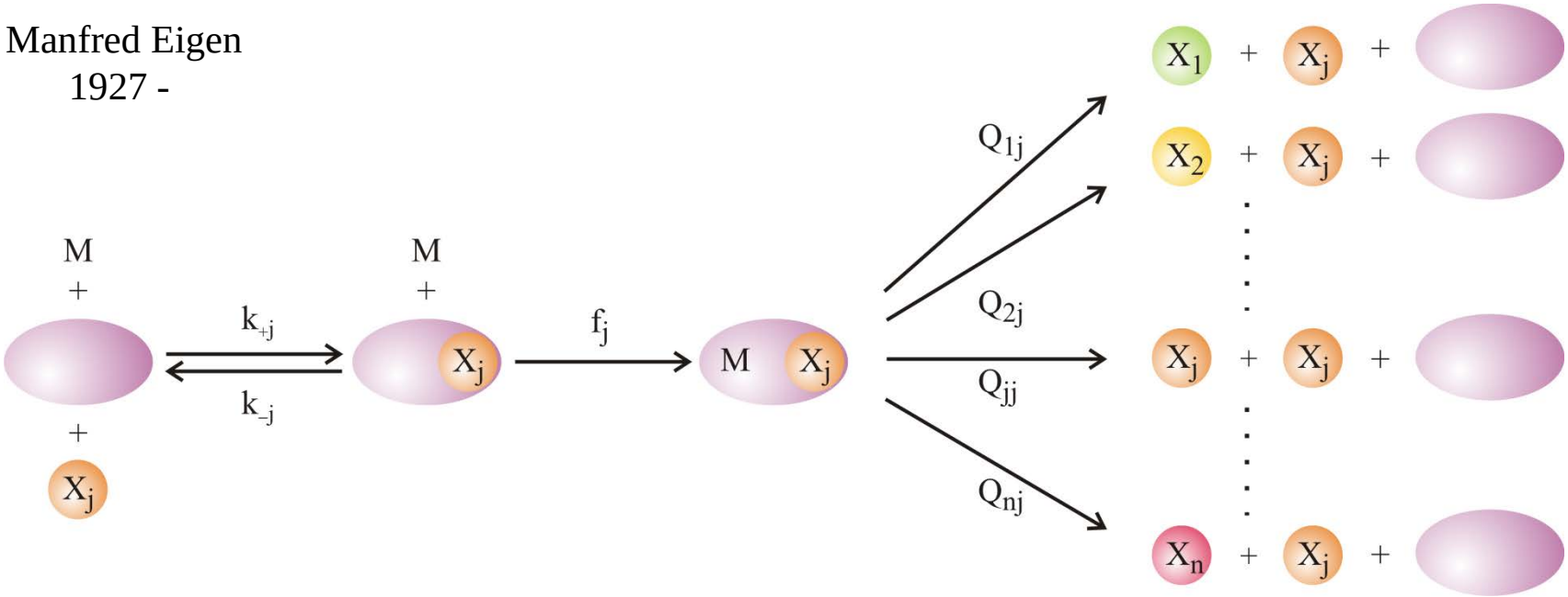

Fig. 9. Serial transfer experiment. Each 0·25 ml standard reaction mixture contained 40 μg of Qβ replicase and $^{32}$P-UTP. The first reaction (0 transfer) was initiated by the addition of 0·2 μg ts-1 (temperature-sensitive RNA) and incubated at 35 °C for 20 min, whereupon 0·02 ml was drawn for counting and 0·02 ml was used to prime the second reaction (first transfer), and so on. After the first 13 reactions, the incubation periods were reduced to 15 min (transfers 14–29). Transfers 30–38 were incubated for 10 min. Transfers 39–52 were incubated for 7 min, and transfers 53–74 were incubated for 5 min. The arrows above certain transfers (0, 8, 14, 29, 37, 53, and 73) indicate where 0·001–0·1 ml of product was removed and used to prime reactions for sedimentation analysis on sucrose. The inset examines both infectious and total RNA. The results show that biologically competent RNA ceases to appear after the 4th transfer (Mills *et al.* 1967).

D.R.Mills, R,L,Peterson, S.Spiegelman, *An extracellular Darwinian experiment with a self-duplicating nucleic acid molecule*. Proc.Natl.Acad.Sci.USA **58** (1967), 217-224

Manfred Eigen
1927 -

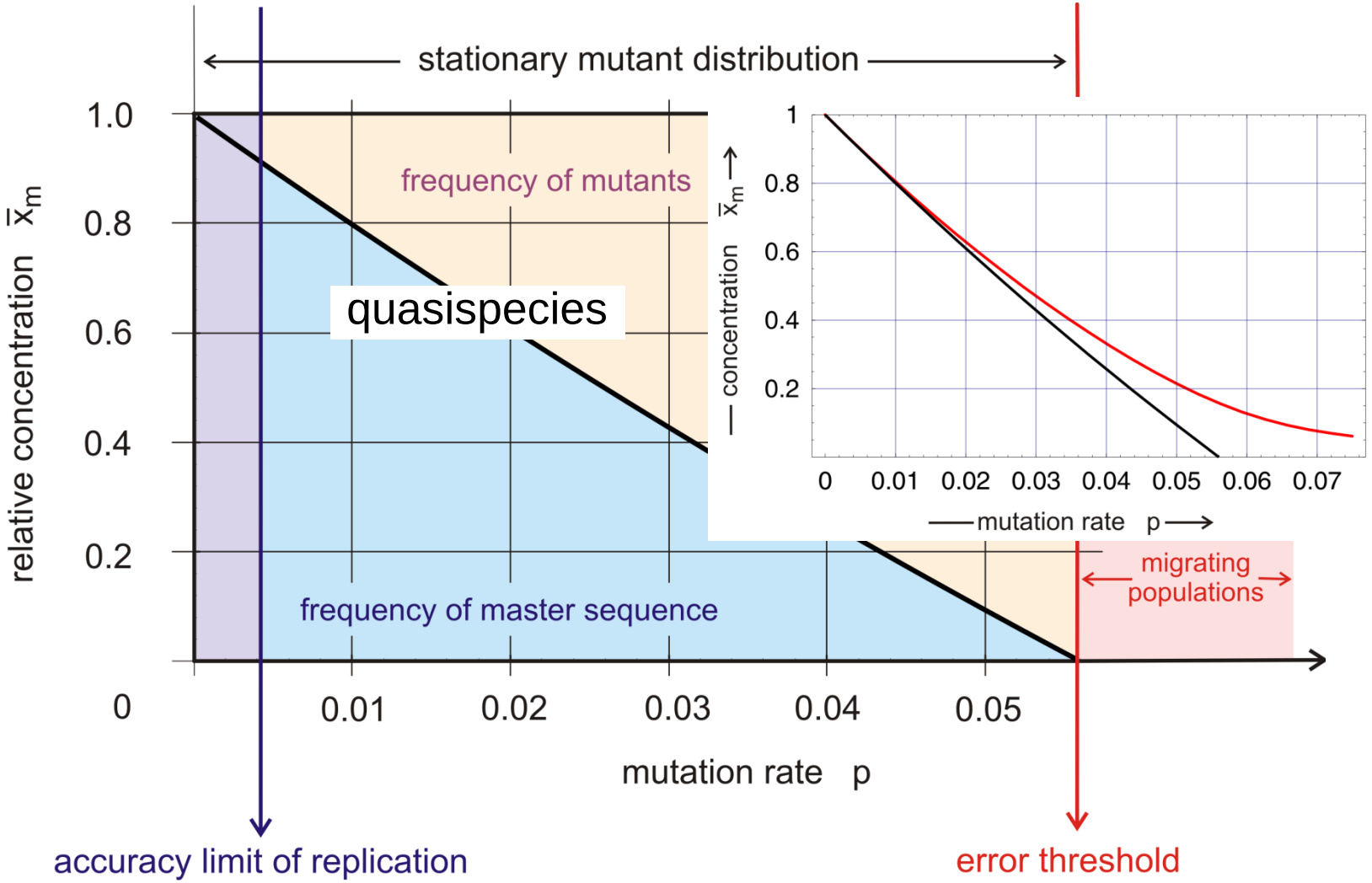
$$\frac{dx_j}{dt} = \sum_{i=1}^{n} W_{ji}\, x_i - x_j\, \Phi\,; \quad j = 1, 2, \ldots, n$$

$$\Phi = \sum_{i=1}^{n} f_i\, x_i \Big/ \sum_{i=1}^{n} x_i$$




Mutation and (correct) replication as parallel chemical reactions

M. Eigen. 1971. *Naturwissenschaften* 58:465,
M. Eigen & P. Schuster.1977. *Naturwissenschaften* 64:541, 65:7 und 65:341




The error threshold in replication and mutation

## Results of the kinetic theory of evolution

1. Not a single "wild type" is selected but a fittest genotype together with its mutant cloud forming a **quasispecies**.

2. Mutation rates are limited by an **error threshold** above which genetic information is unstable.

3. For a given replication machinery the error threshold sets a limit to the length of genomes.

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Virus Research 107 (2005) 115–116
Virus Research
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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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Esteban Domingo
1943 -

Application of quasispecies theory to the fight against viruses

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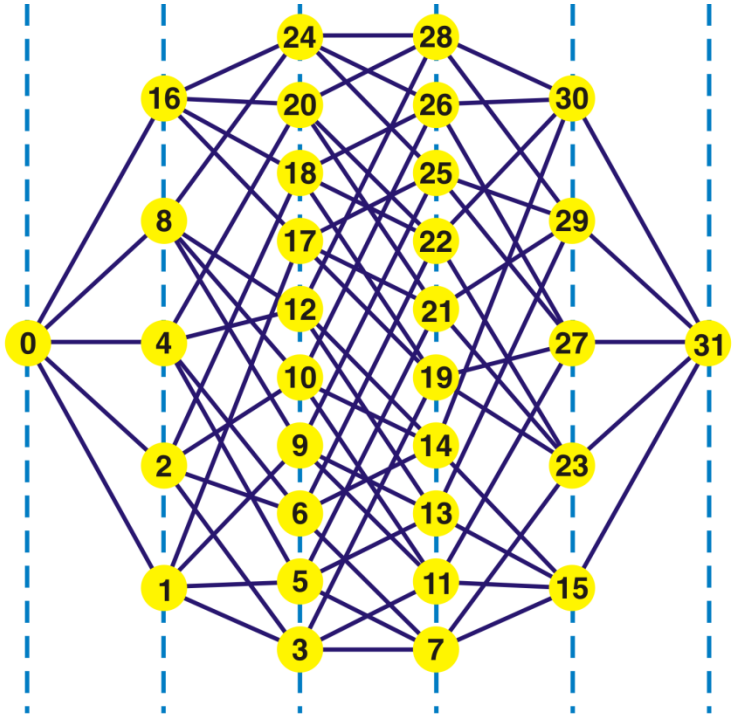



**Binary sequences are encoded by their decimal equivalents:**

**C = 0 and G = 1, for example,**

**"0" ≡ 00000 = CCCCC,**

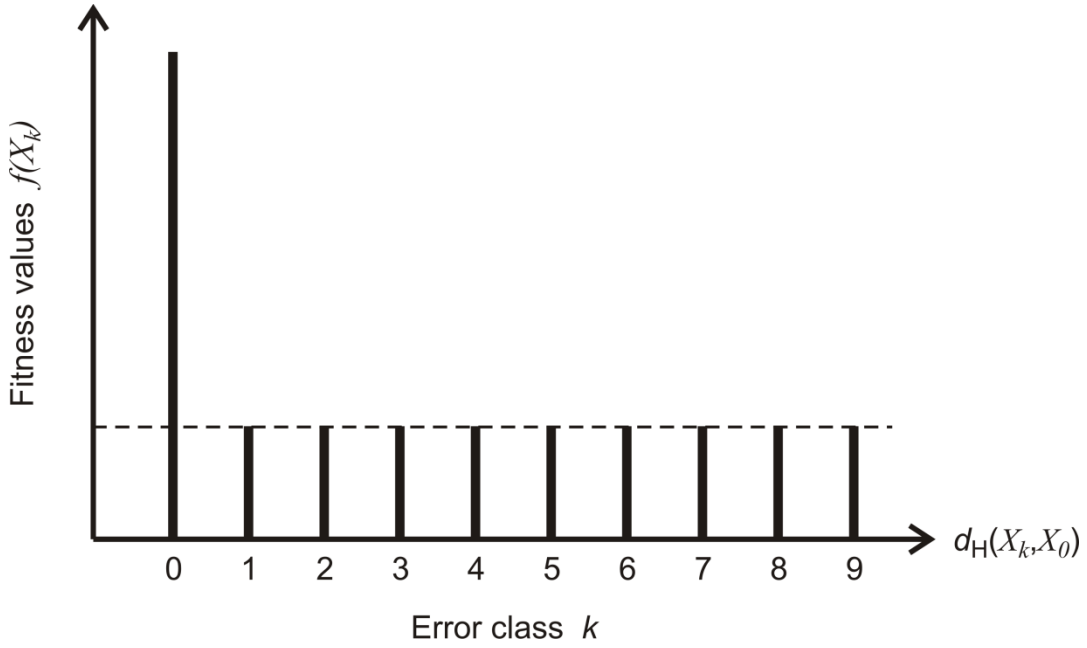
**"14" ≡ 01110 = CGGGC,**

**"29" ≡ 11101 = GGGCG, etc.**

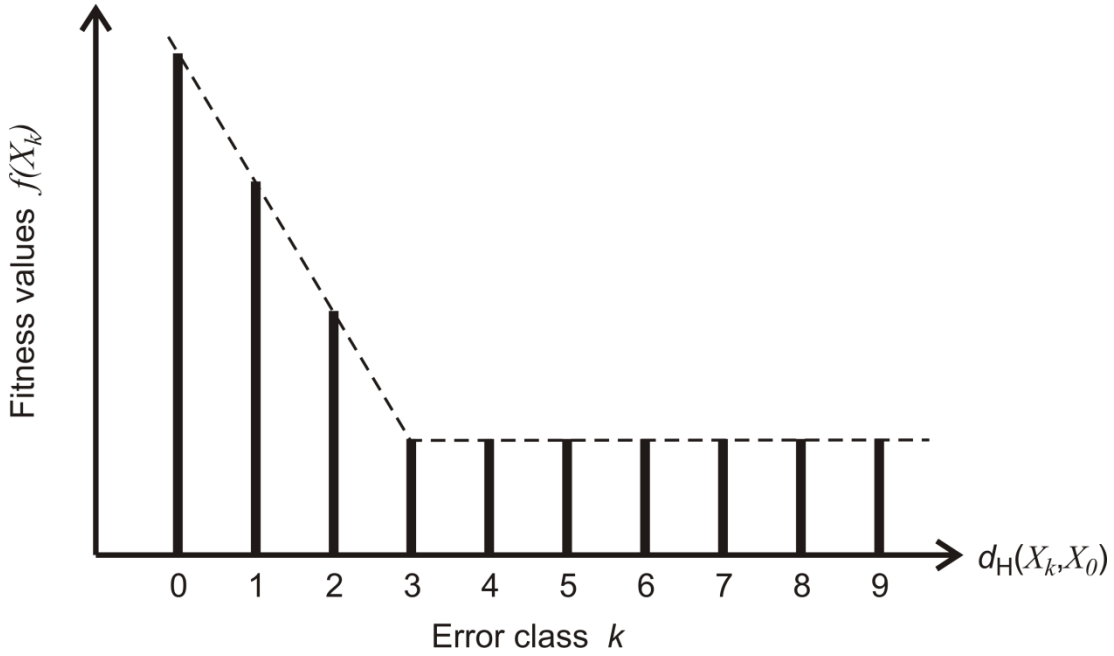
Concentrations of entire error classes: $[\Gamma_k] = y_k(p)\,,\ k = 0, 1, \ldots, n$

$$y_k(p) = \sum_{i=1,\ d_{\mathrm{H}}(\mathsf{X}_i,\mathsf{X}_k)=k}^{N} x_i(p)\,, \quad |\Gamma_k| = \binom{n}{k}$$

single peak landscape




step linear landscape




Model fitness landscapes I

Biophysical Chemistry 16 (1982) 329–345
Elsevier Biomedical Press

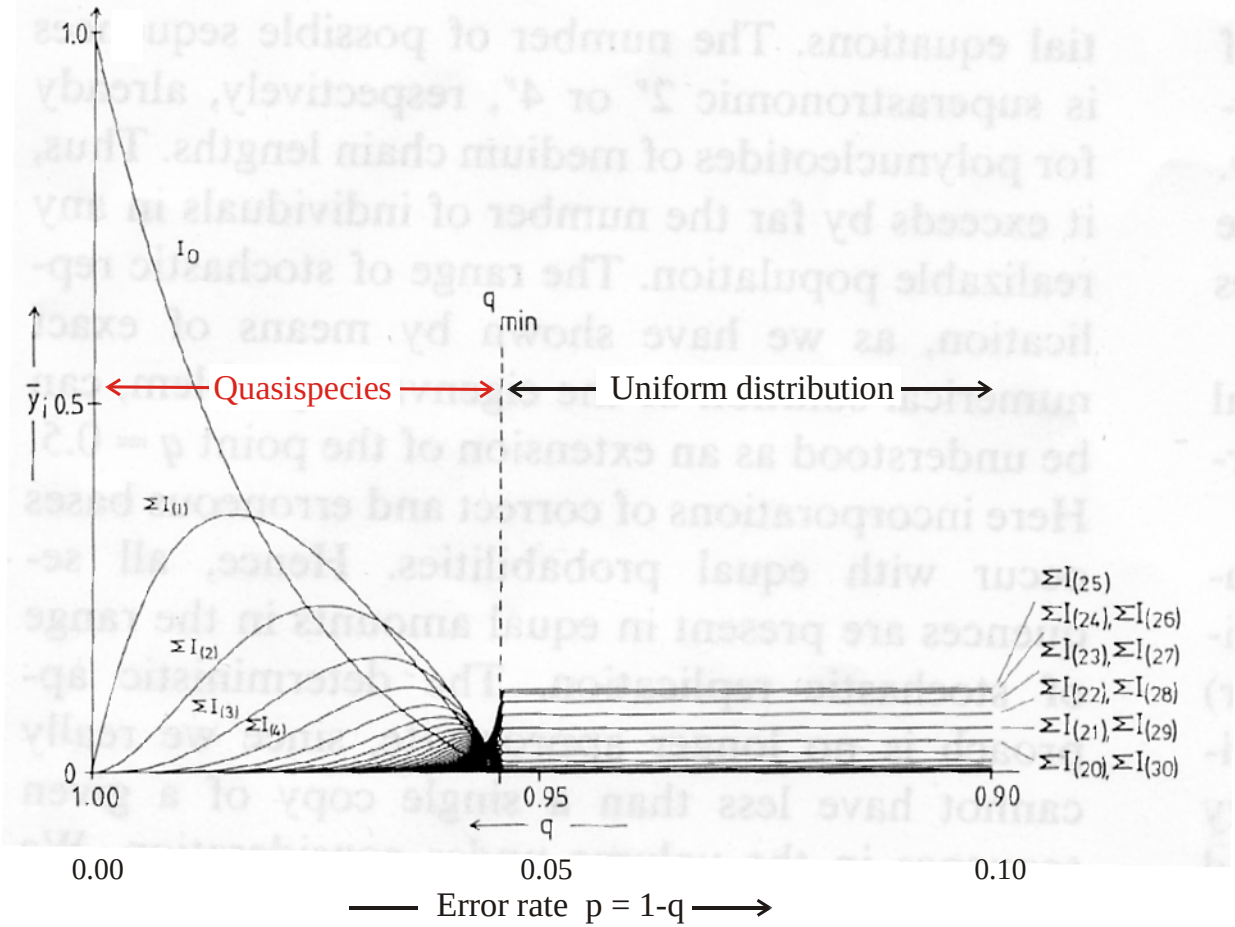


**SELF-REPLICATION WITH ERRORS**

**A MODEL FOR POLYNUCLEOTIDE REPLICATION ****

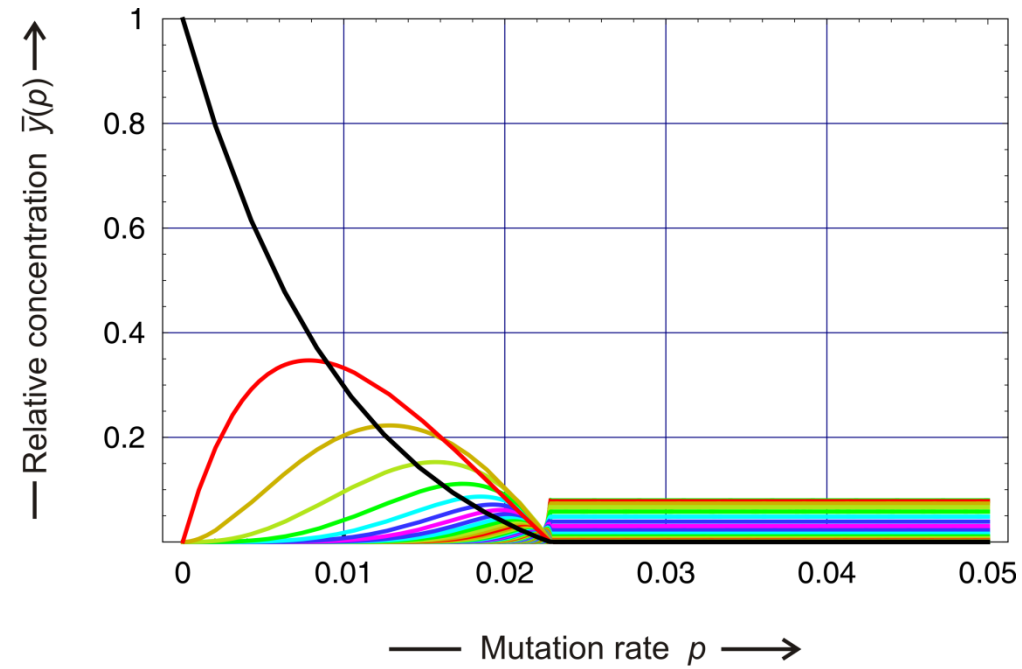
Jörg SWETINA and Peter SCHUSTER *

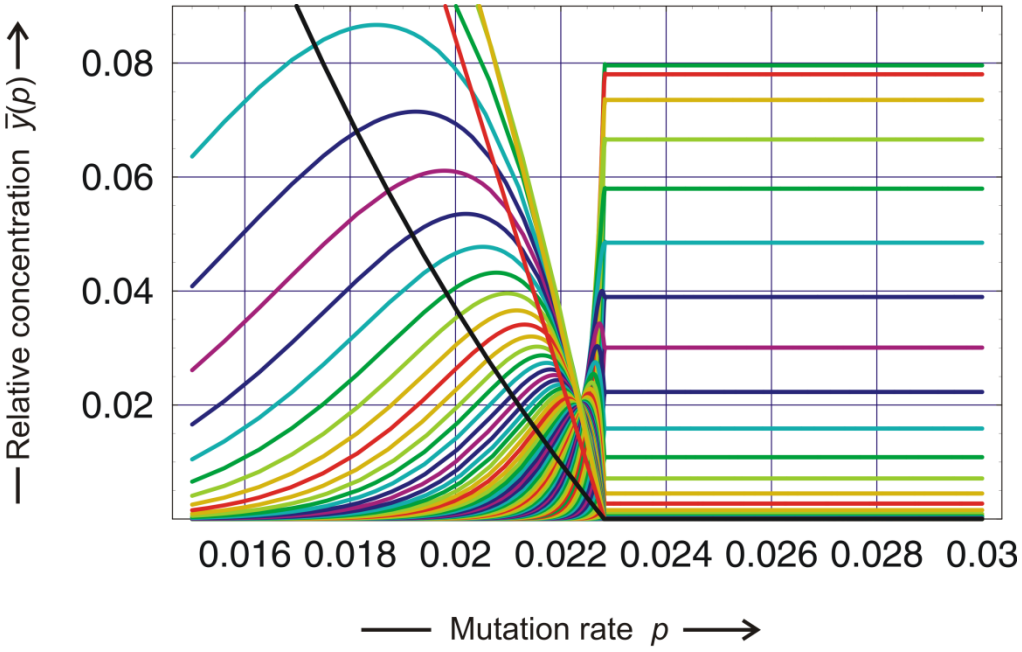
*Institut für Theoretische Chemie und Strahlenchemie der Universität, Währingerstraße 17, A-1090 Wien, Austria*



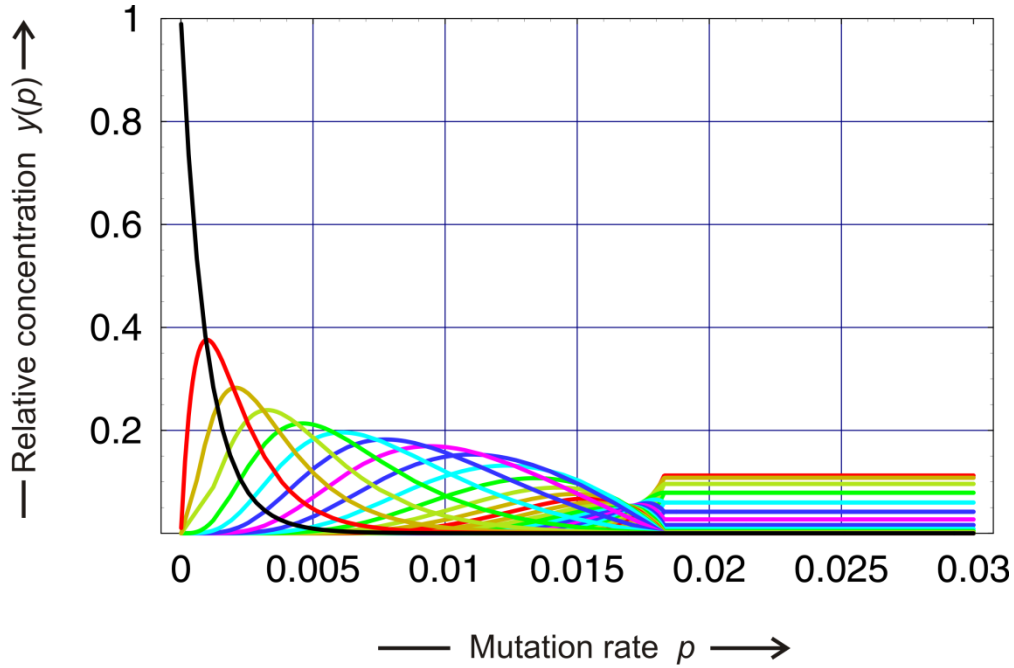

Stationary population or quasispecies as a function of the mutation or error rate *p*

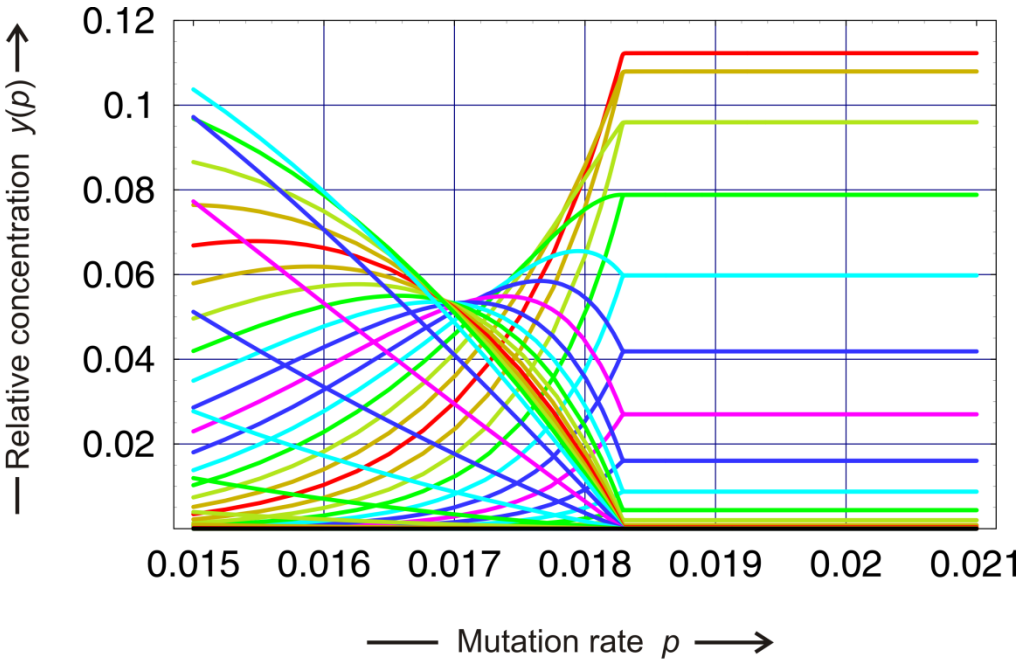
Error threshold on the single peak landscape



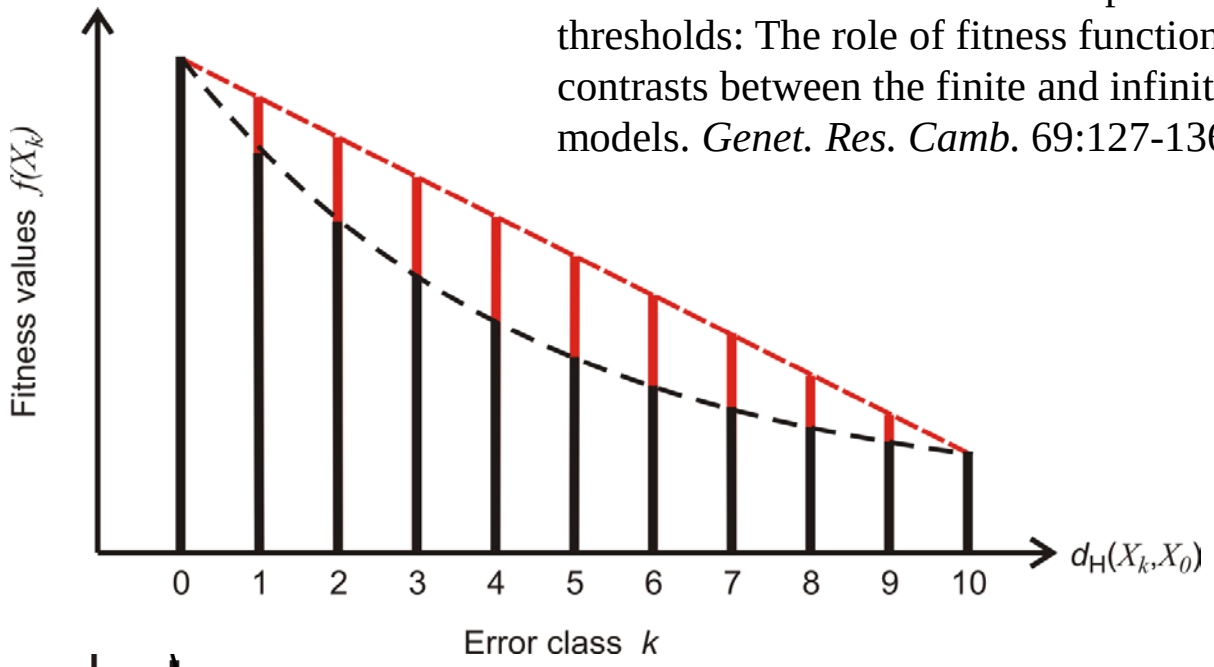
Error threshold on the step linear landscape



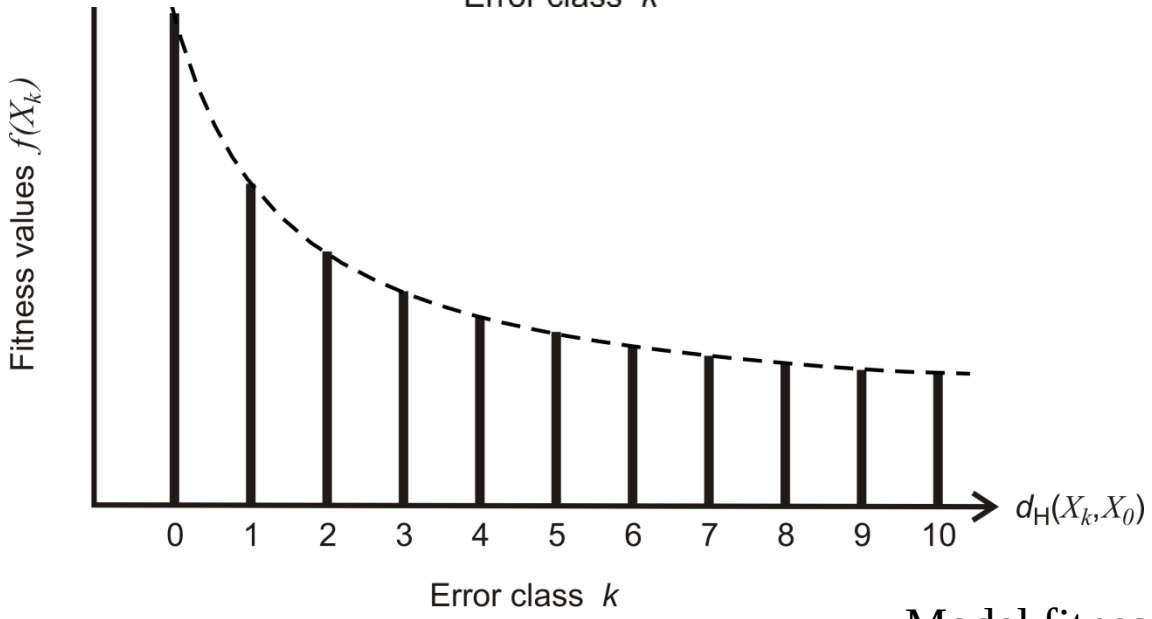



Thomas Wiehe. 1997. Model dependency of error thresholds: The role of fitness functions and contrasts between the finite and infinite sites models. *Genet. Res. Camb.* 69:127-136

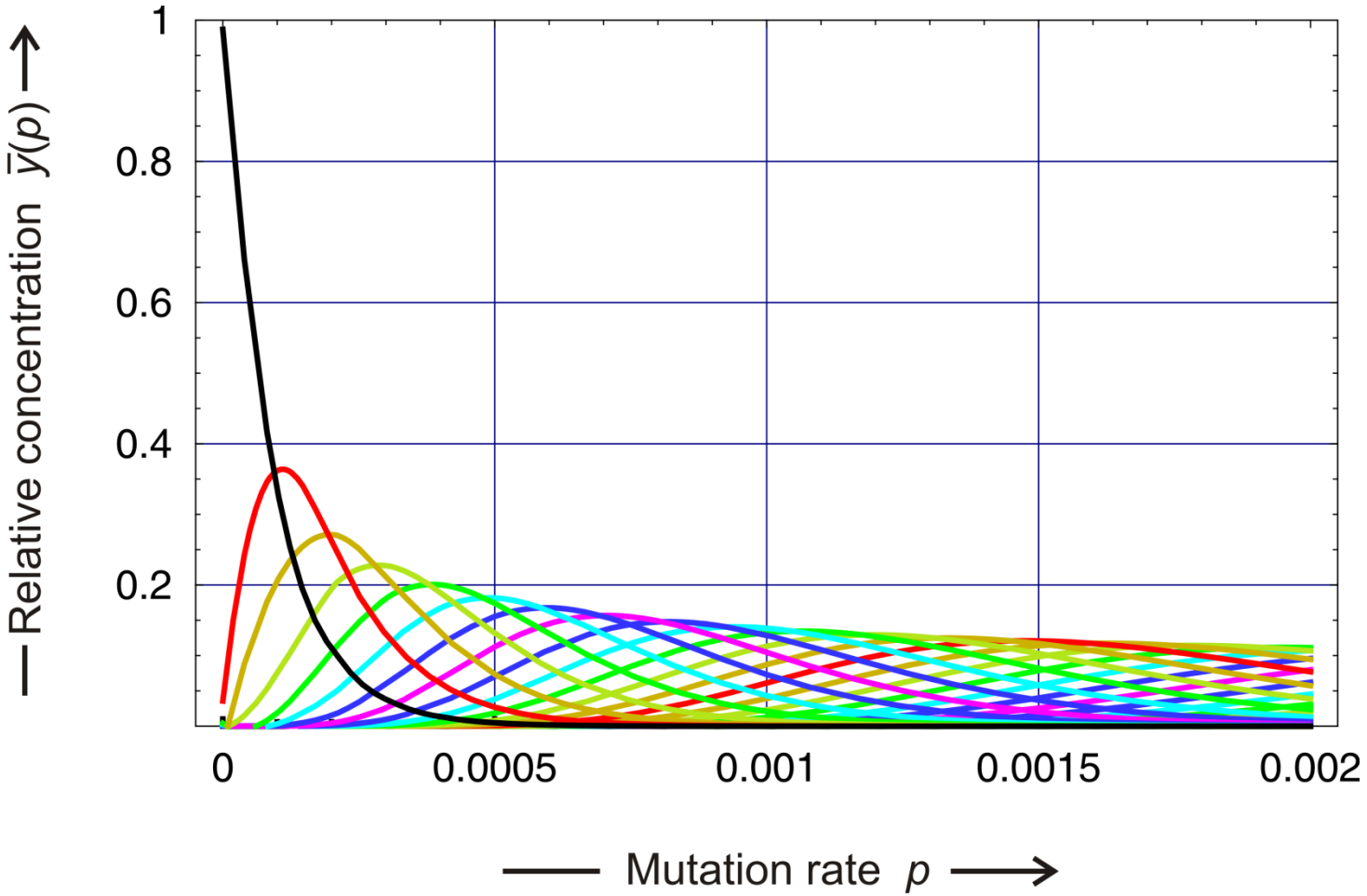
linear and multiplicative




hyperbolic

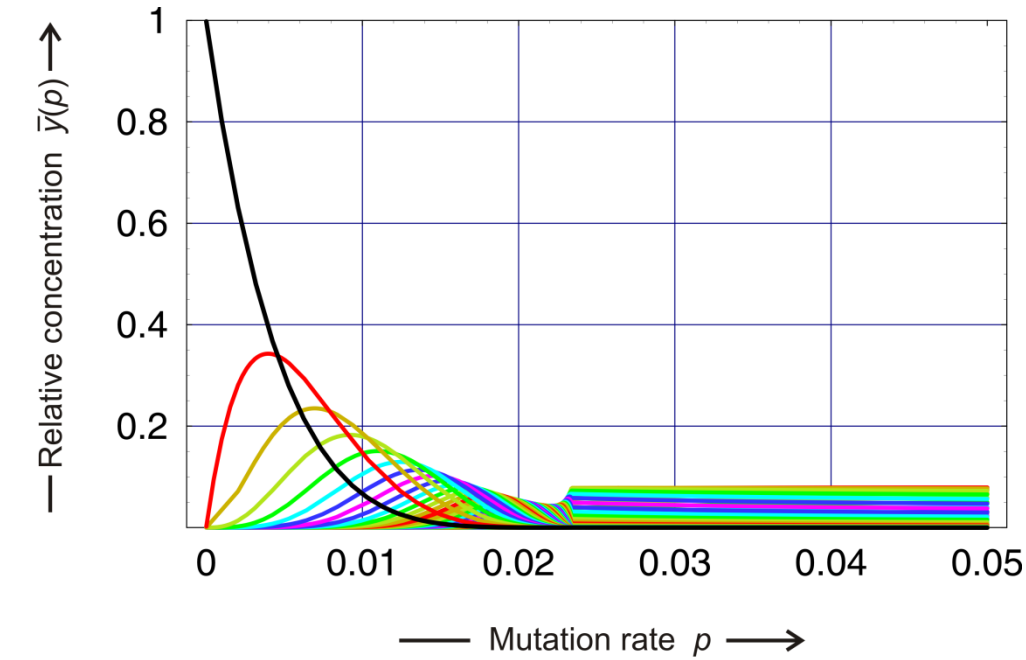


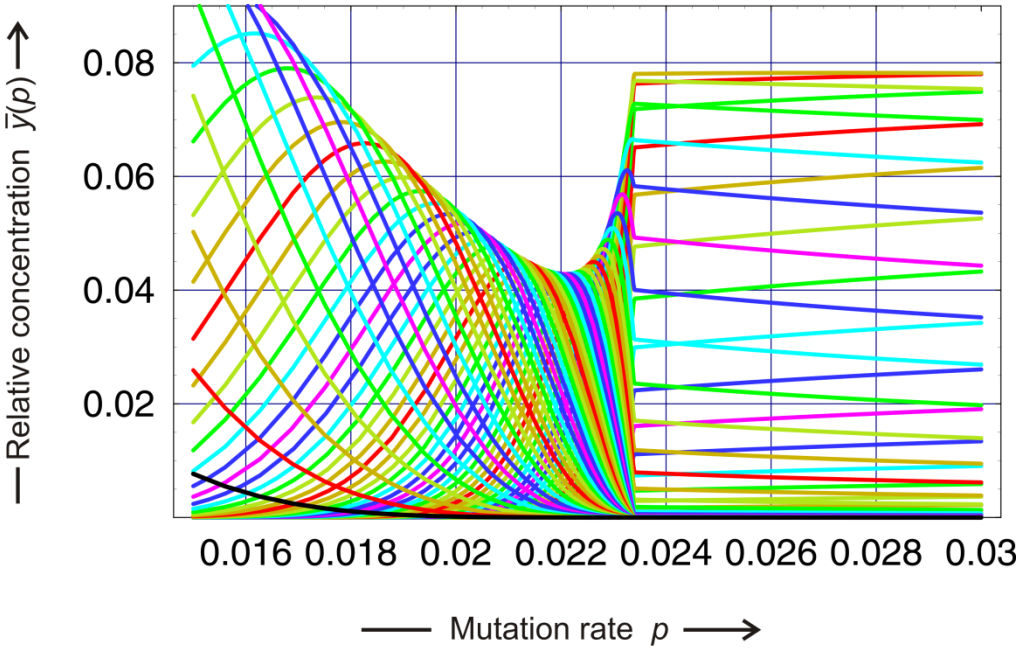

Model fitness landscapes II




The linear fitness landscape shows no error threshold

Error threshold on the hyperbolic landscape



The error threshold can be separated into three phenomena:

1. **Steep decrease** in the concentration of the master sequence to very small values.

2. **Sharp change** in the stationary concentration of the quasispecies distribuiton.

3. Transition to the **uniform distribution** at small mutation rates.

**All three phenomena coincide for the quasispecies on the single peak fitness lanscape.**

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## Fitness landscapes became experimentally accessible!

**Protein landscapes**: Yuuki Hayashi, Takuyo Aita, Hitoshi Toyota, Yuzuru Husimi, Itaru Urabe, Tetsuya Yomo. 2006. Experimental rugged fitness landscape in protein seqeunce space. *PLoS One* 1:e96.

**RNA landscapes**: Sven Klussman, Ed. 2005. The aptamer handbook. Wiley-VCh, Weinheim (Bergstraße), DE.
Jason N. Pitt, Adrian Ferré-D'Amaré. 2010. Rapid construction of empirical RNA fitness landscapes. *Science* 330:376-379.

**RNA viruses**: Esteban Domingo, Colin R. Parrish, John J. Holland, Eds. 2007. Origin and evolution of viruses. Second edition. Elesvier, San Diego, CA.

**Retroviruses**: Roger D. Kouyos, Gabriel E. Leventhal, Trevor Hinkley, Mojgan Haddad, Jeannette M. Whitcomb, Christos J. Petropoulos, Sebastian Bonhoeffer. 2012. Exploring the complexity of the HIV-I fitness landscape. *PLoS Genetics* 8:e1002551

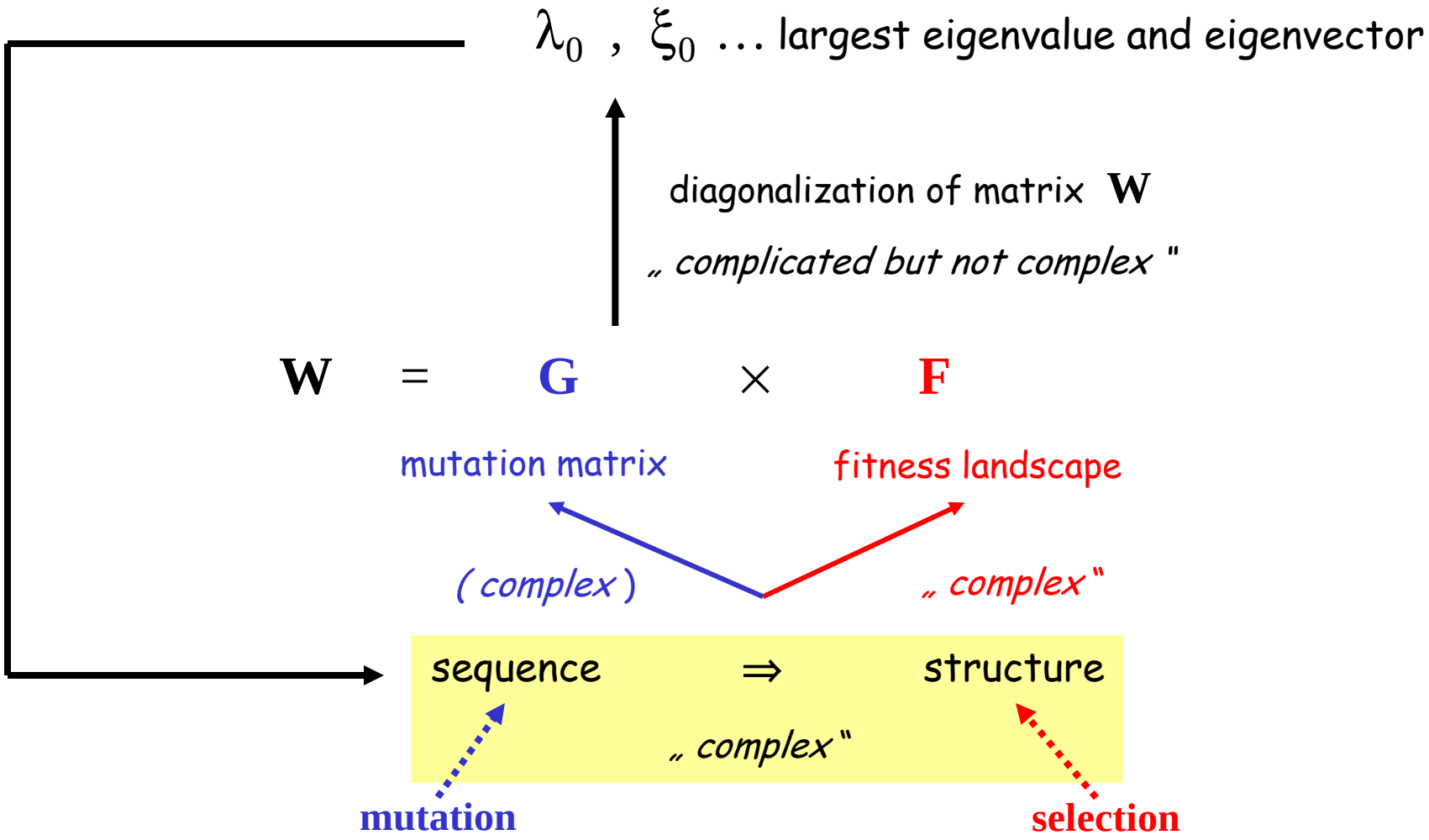
## Realistic fitness landscapes

1. Ruggedness: nearby lying genotypes may develop into very different phenotypes

2. Neutrality: many different genotypes give rise to phenotypes with identical selection behavior

3. Combinatorial explosion: the number of possible genomes is prohibitive for systematic searches

**Facit**: Any successful and applicable theory of molecular evolution must be able to predict evolutionary dynamics from a small or at least in practice measurable number of fitness values.




Complexity in molecular evolution

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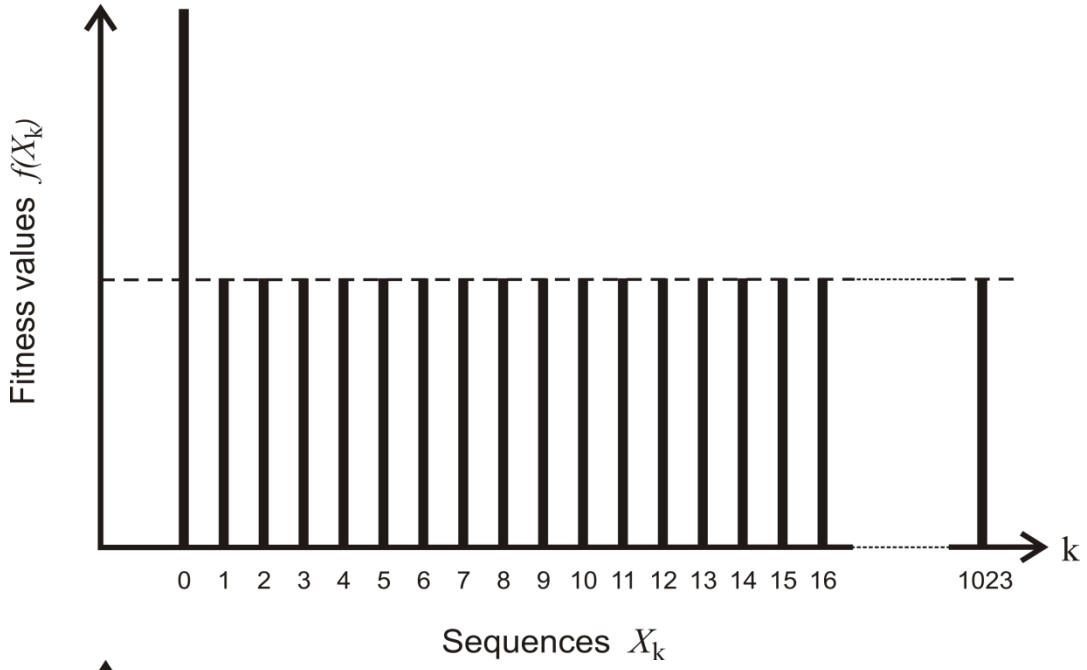
single peak landscape

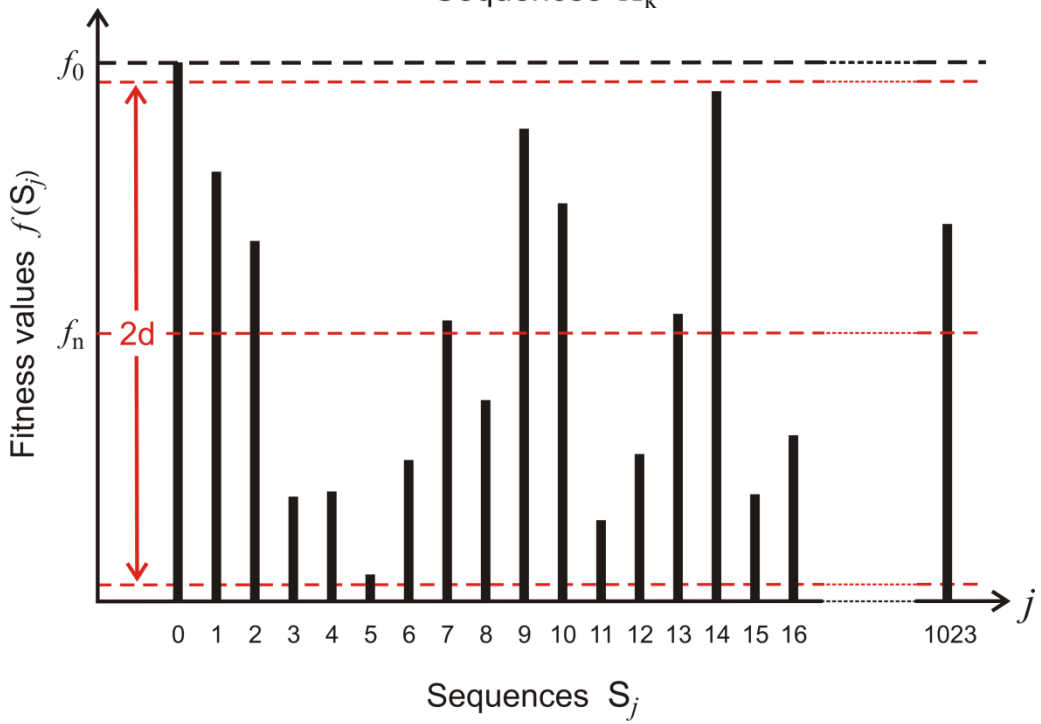



$$f(\mathsf{S}_j) = f_n + 2\,d\,(f_0 - f_n)\left(\eta_j^{(s)} - 0.5\right)$$

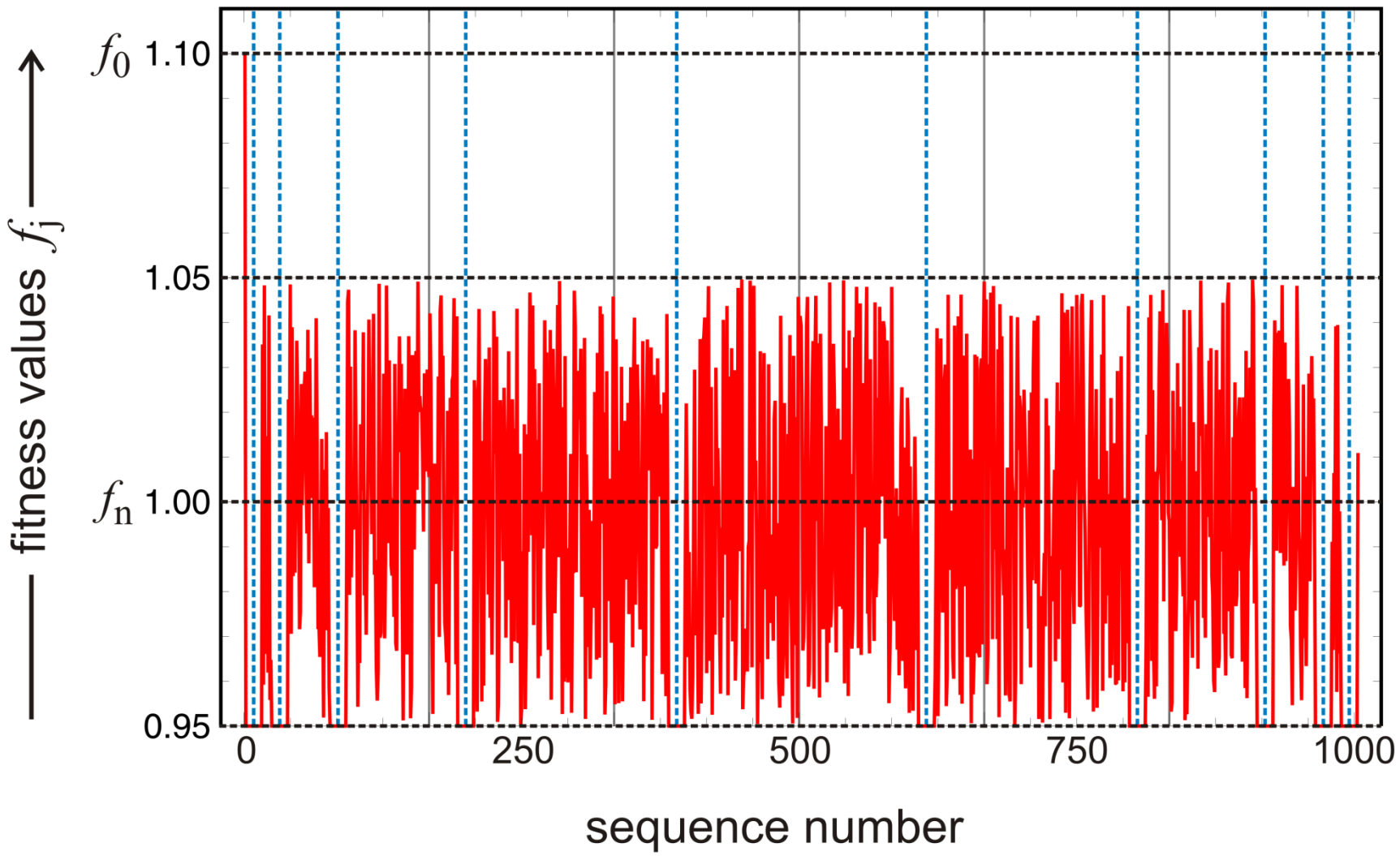
$$j = 1, 2, \ldots, N\,;\; j \neq m\,,$$

$\eta$ ... random number ; $s$ ... seeds

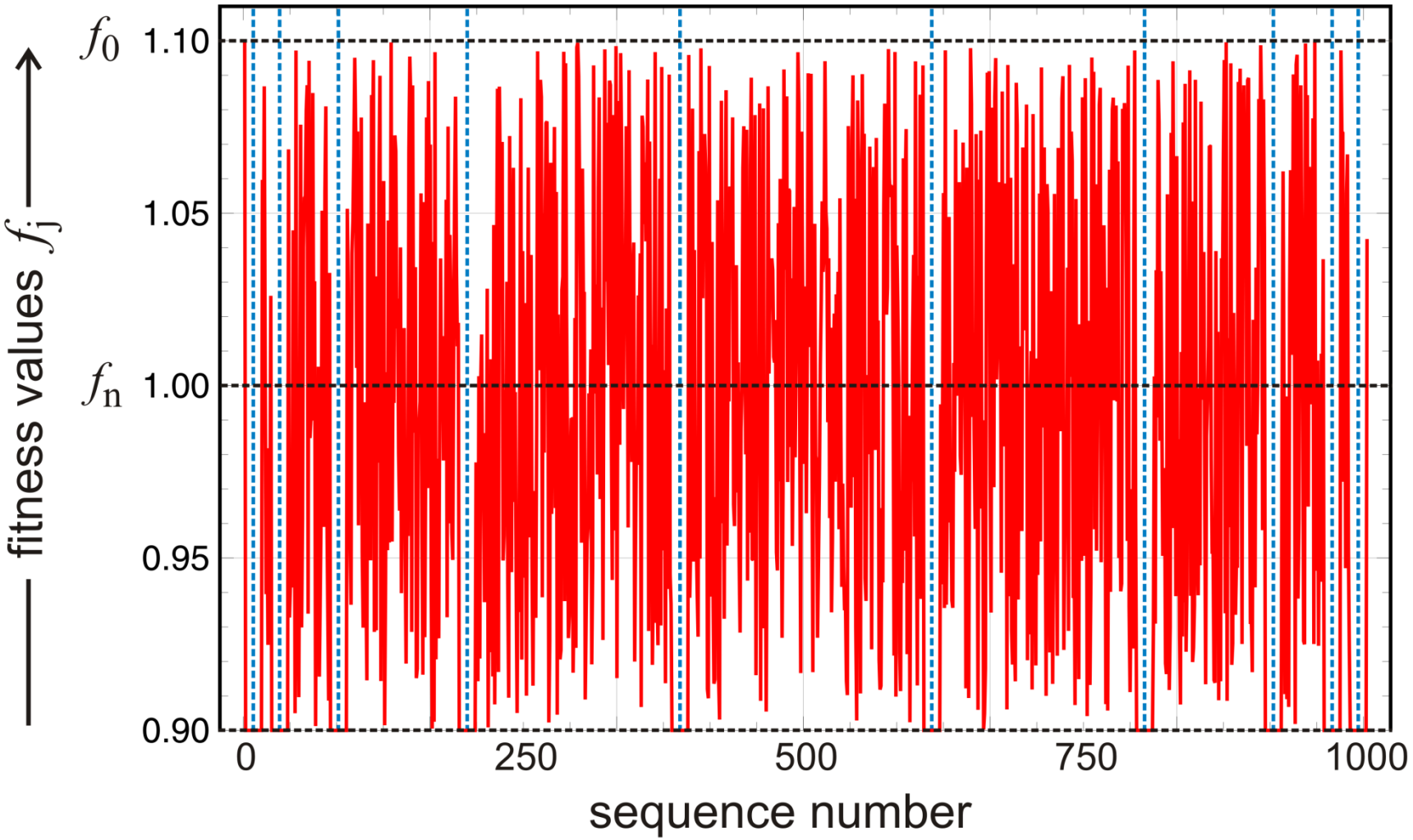
„realistic“ landscape



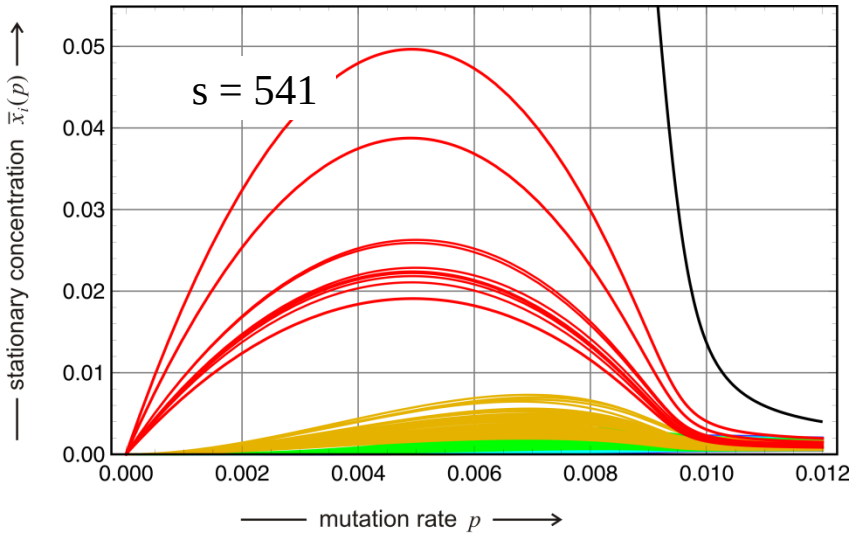

Rugged fitness landscapes
over individual binary sequences
with n = 10

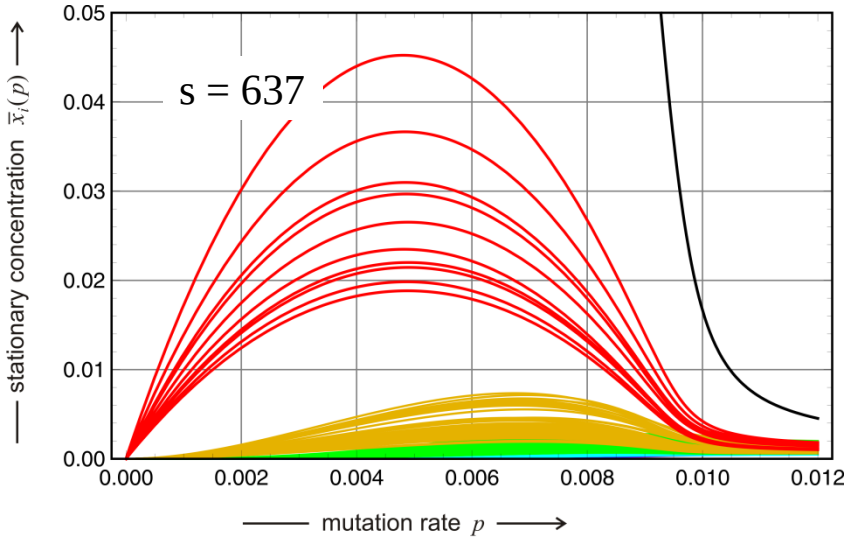


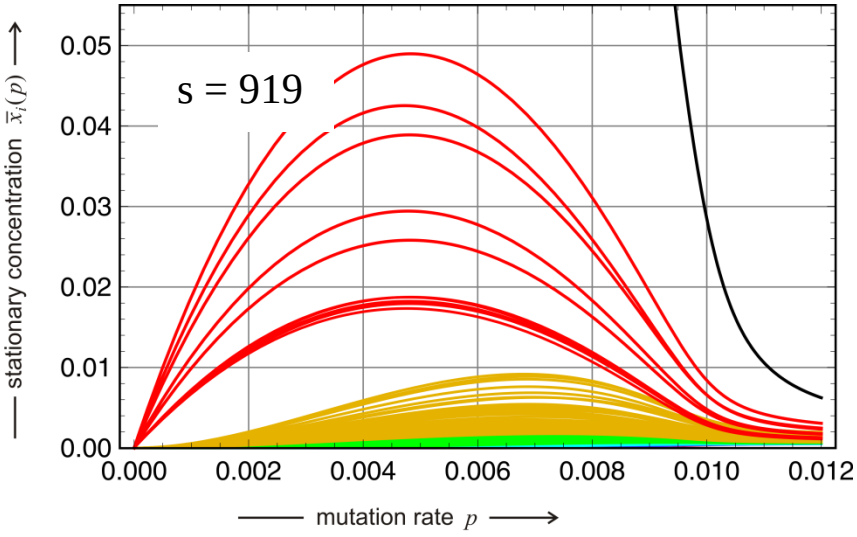

Random distribution of fitness values: $d = 0.5$ and $s = 919$




Random distribution of fitness values: $d$ = 1.0 and $s$ = 637

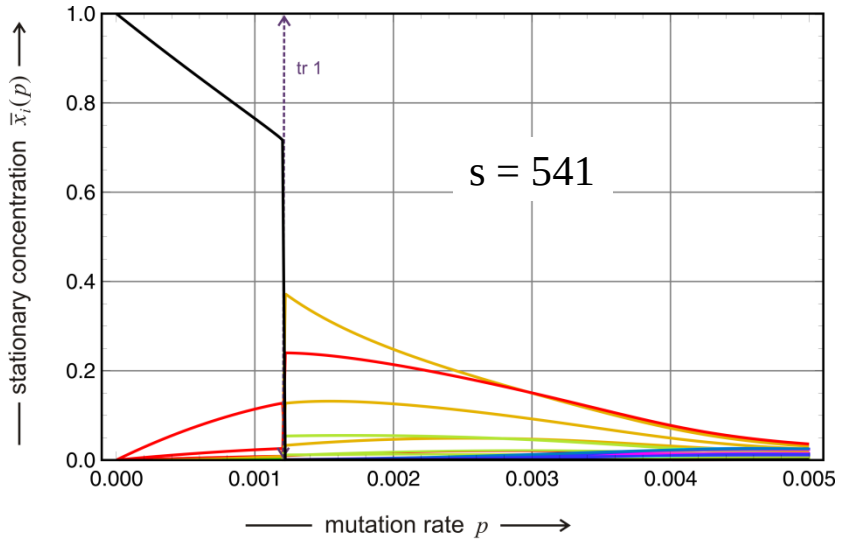


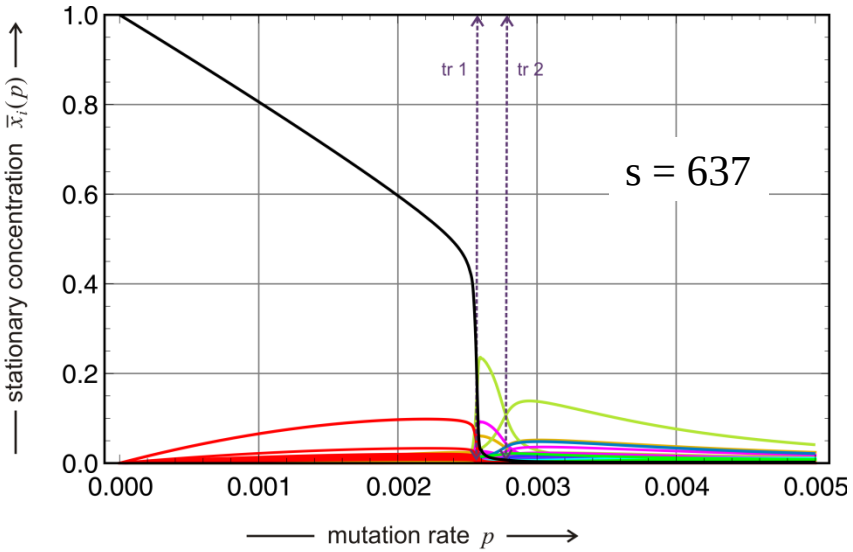



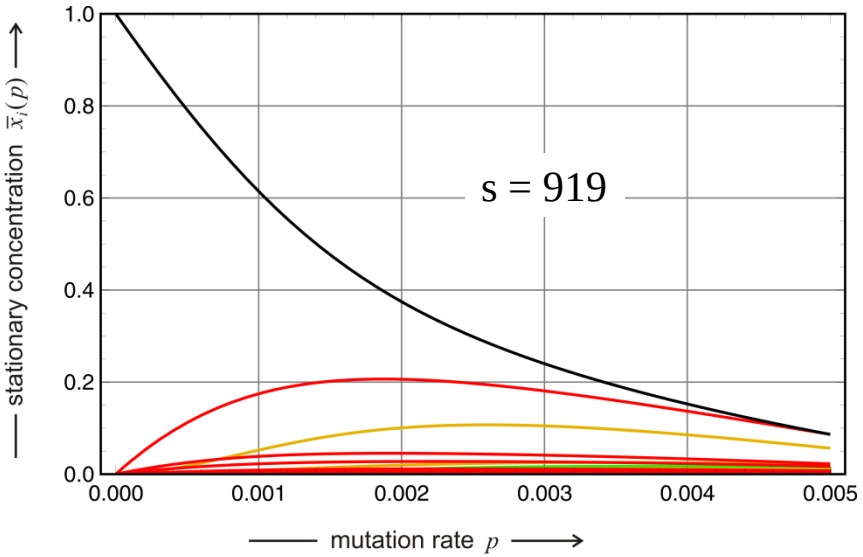




Error threshold on ‚realistic' landscapes

n = 10, $f_0 = 1.1$, $f_n = 1.0$, d = 0.5

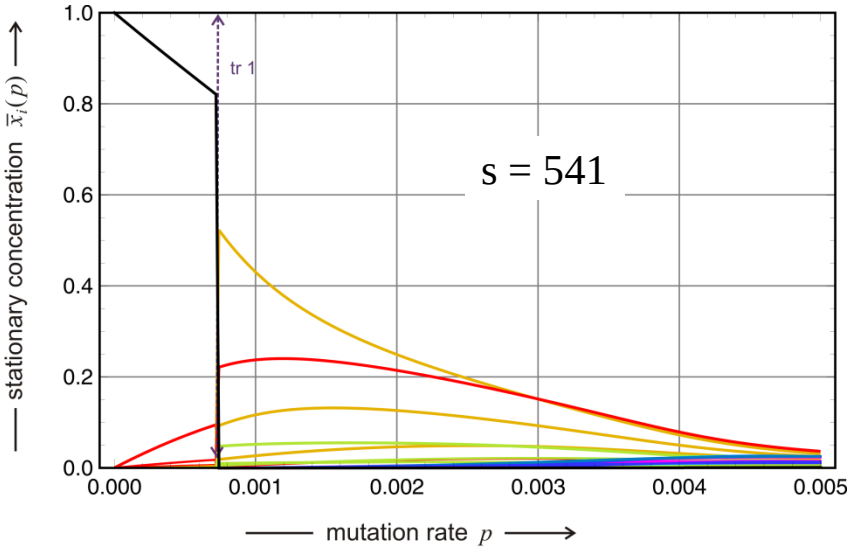


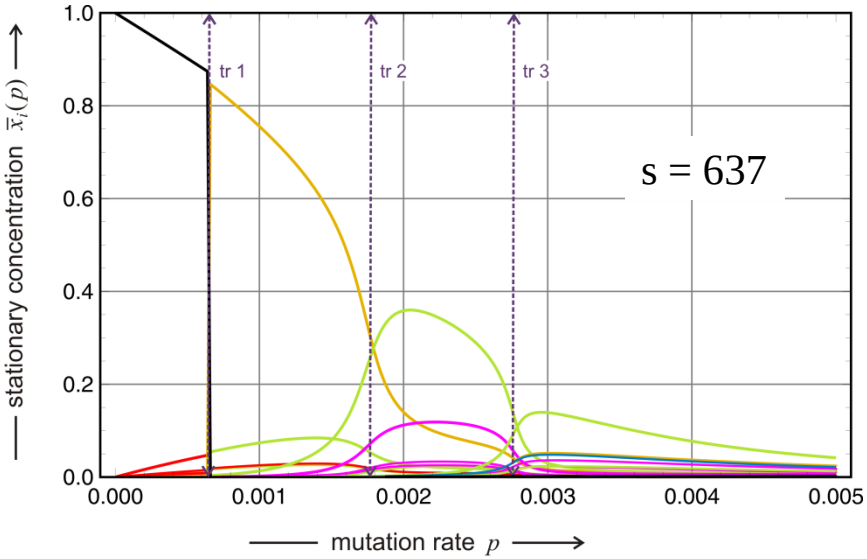



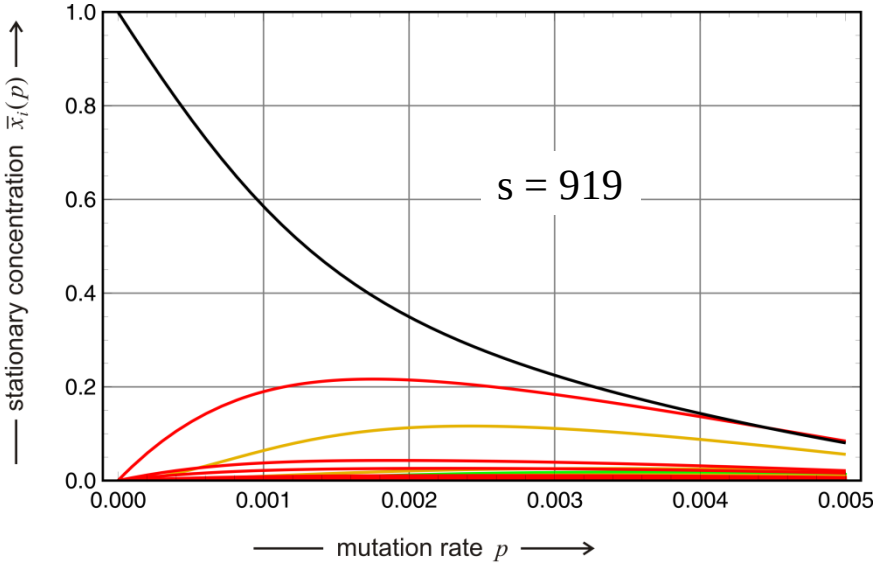




Error threshold on ‚realistic' landscapes

n = 10, $f_0 = 1.1$, $f_n = 1.0$, d = 0.995



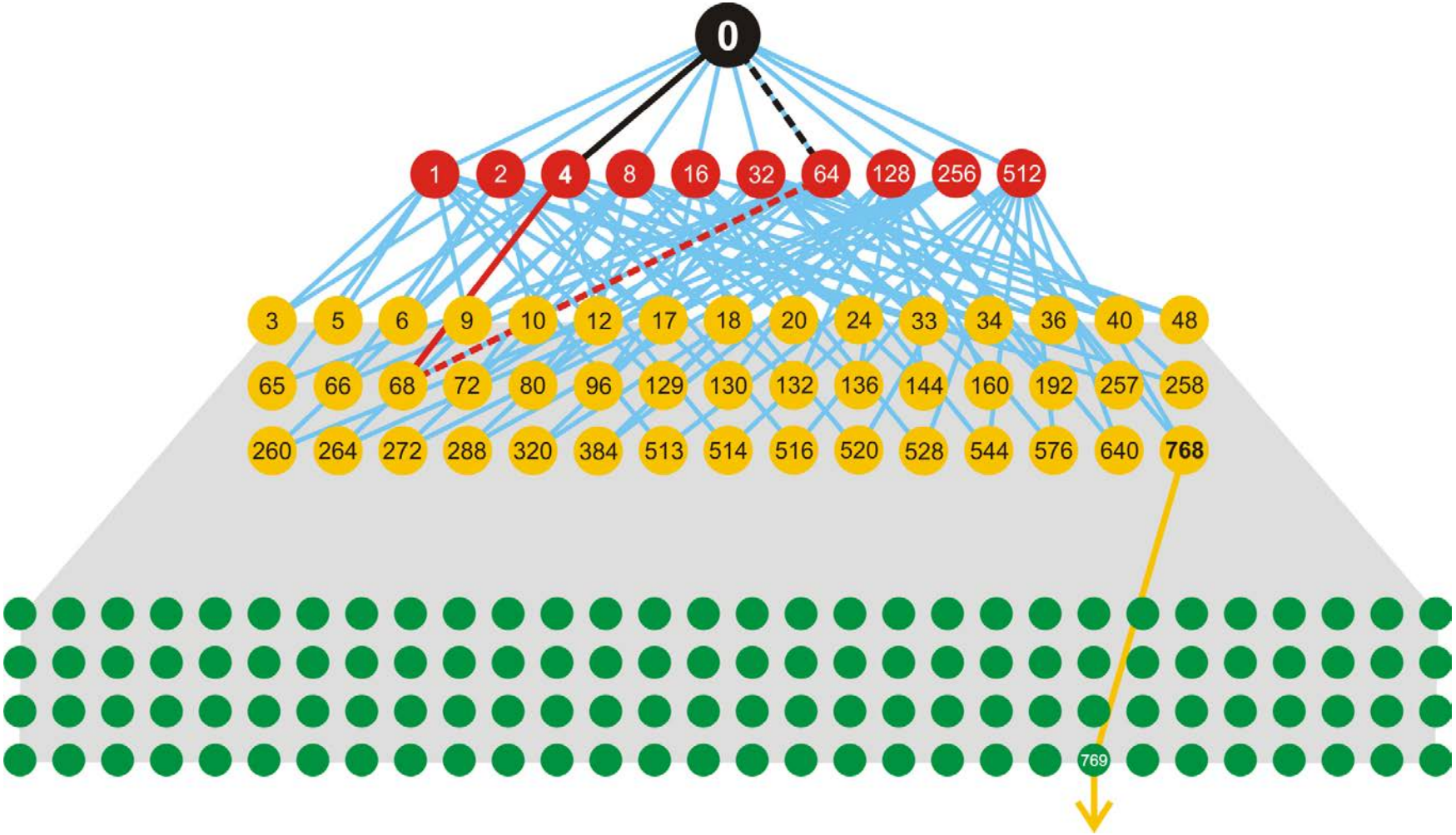

Error threshold on ‚realistic' landscapes

n = 10, $f_0 = 1.1$, $f_n = 1.0$, d = 1.0

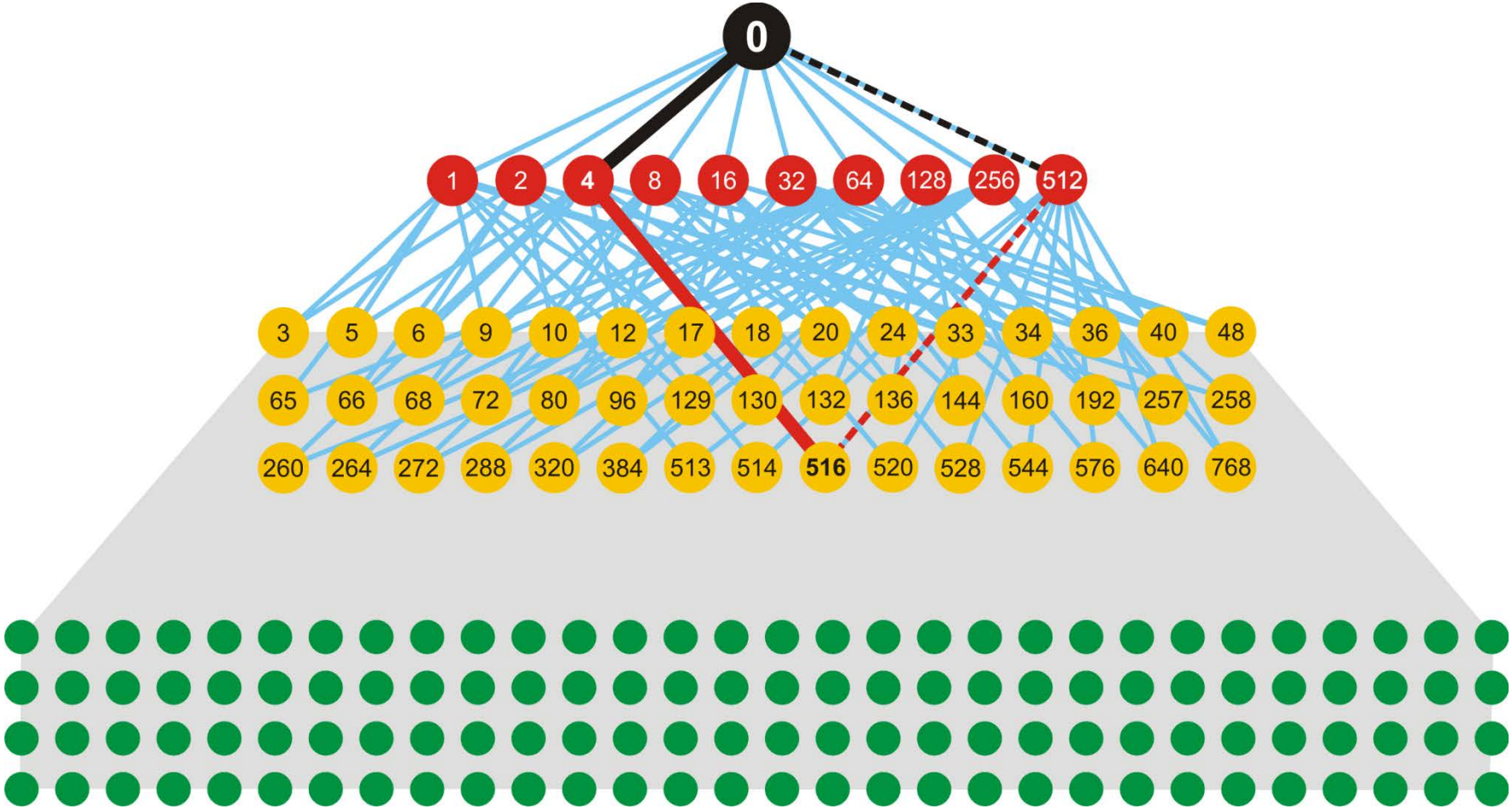
**Two questions**:

1. Can we predict evolutionary dynamics of quasispecies from fitness landscapes?

2. What is the evolutionary consequence of the occurrence of mutationally stable and unstable quasispecies?




Determination of the dominant mutation flow: $d = 1$ , $s = 613$




Determination of the dominant mutation flow: $d = 1$ , $s = 919$

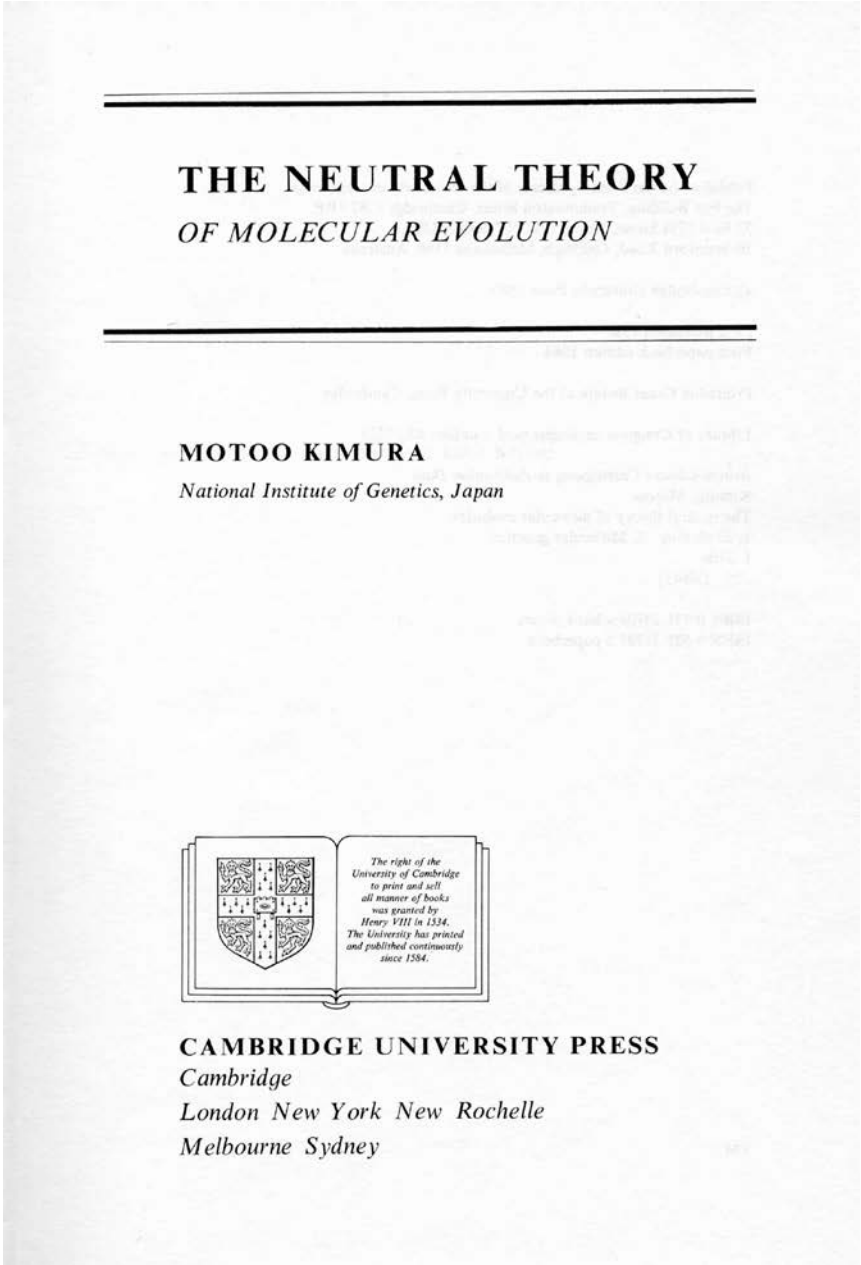
1. History of „fitness landscape“
2. Molecular biology of replication
3. Simple landscapes
4. Landscapes revisited
5. „Realistic“ landscapes
6. **Neutrality in evolution**
7. Perspectives

Motoo Kimura, 1924 - 1994

## Motoo Kimura's population genetics of neutral 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*

MOTOO KIMURA

*National Institute of Genetics, Japan*

The right of the University of Cambridge to print and sell all manner of books was granted by Henry VIII in 1534. The University has printed and published continuously since 1584.

CAMBRIDGE UNIVERSITY PRESS

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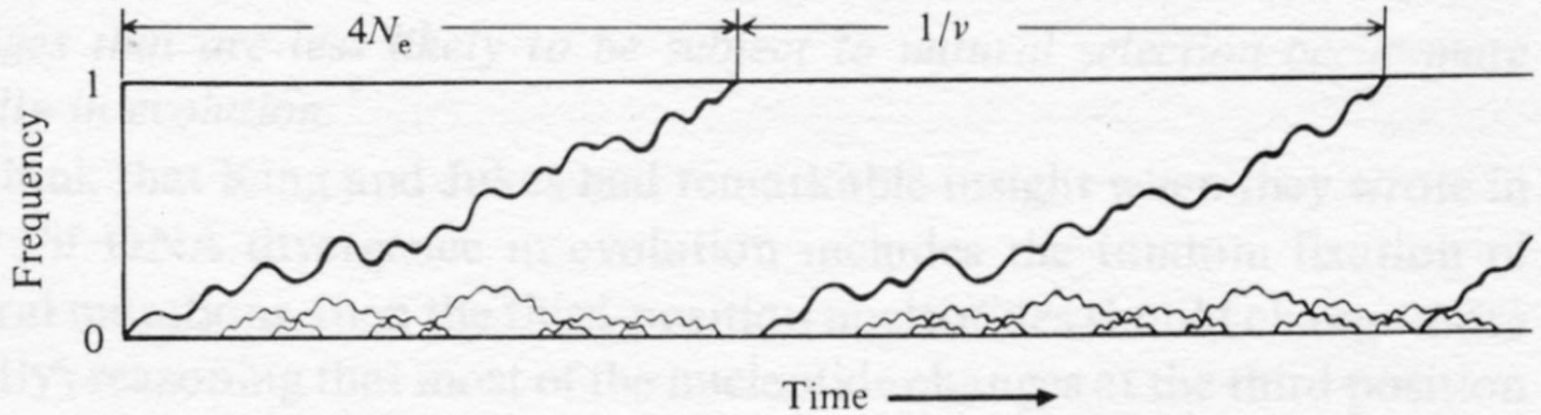
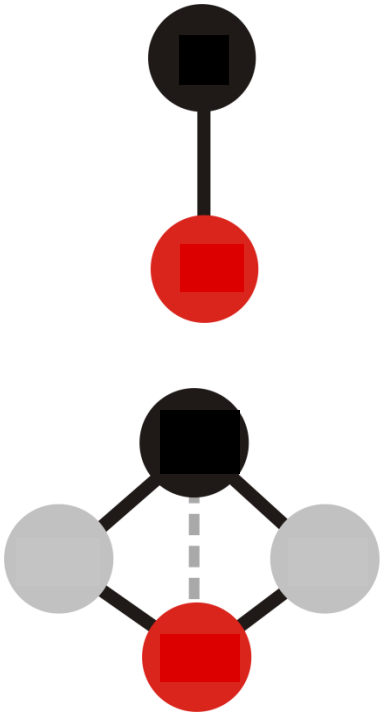
Fig. 3.1. Behavior of mutant genes following their appearance in a finite population. Courses of change in the frequencies of mutants destined to fixation are depicted by thick paths. $N_e$ stands for the effective population size and $v$ is the mutation rate.




Motoo Kimura

Is the Kimura scenario correct for frequent mutations?



$\mathbf{d_H = 1}$

$$\lim_{p\to 0} x_1(p) = x_2(p) = 0.5$$

$\mathbf{d_H = 2}$

$$\lim_{p\to 0} x_1(p) = \alpha/(1+\alpha)$$

$$\lim_{p\to 0} x_2(p) = 1/(1+\alpha)$$

$\mathbf{d_H \geq 3}$

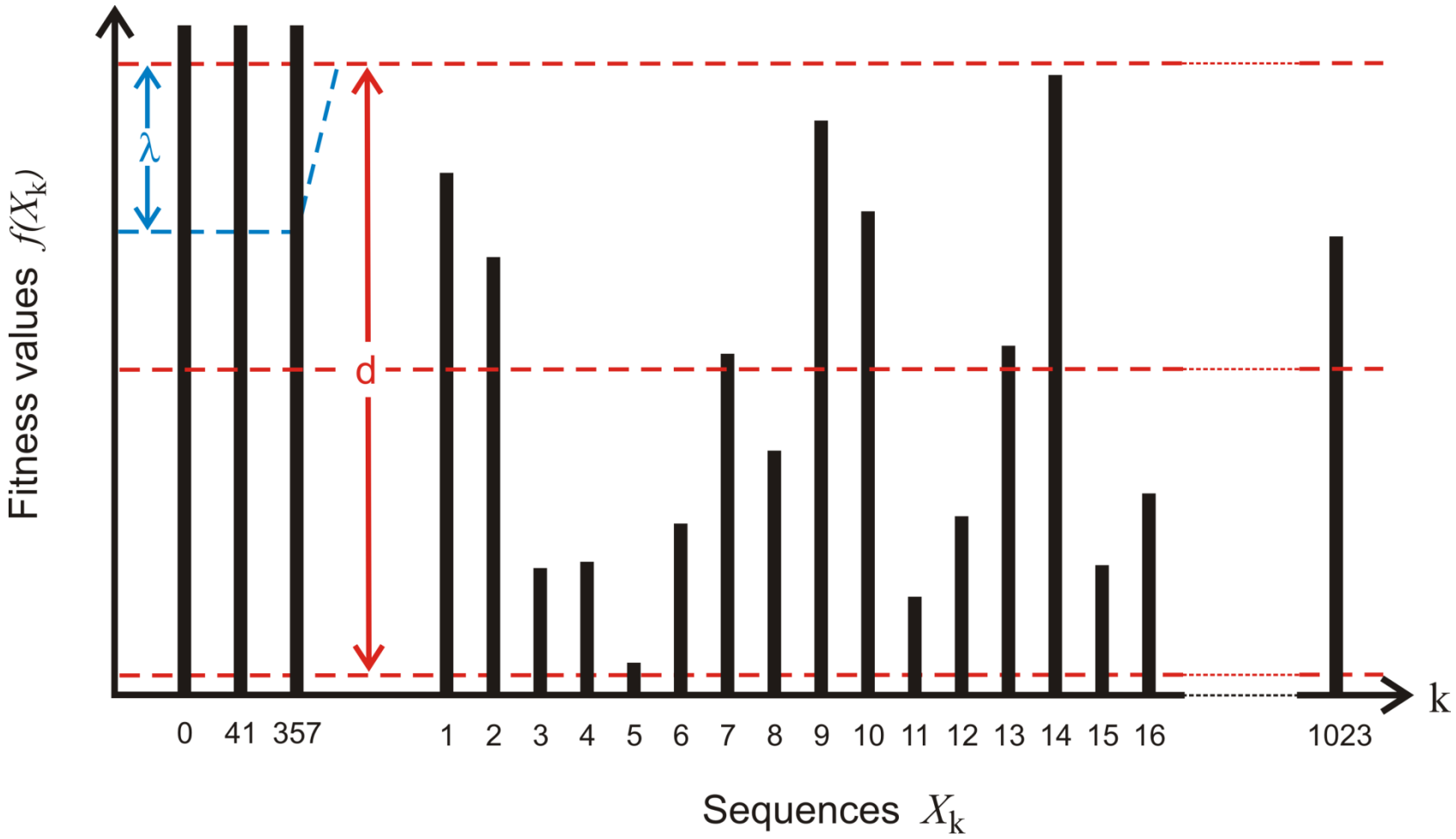
$$\lim_{p\to 0} x_1(p) = 1, \lim_{p\to 0} x_2(p) = 0 \quad \text{or}$$

$$\lim_{p\to 0} x_1(p) = 0, \lim_{p\to 0} x_2(p) = 1$$

Pairs of neutral sequences in replication networks

Random fixation in the sense of Motoo Kimura

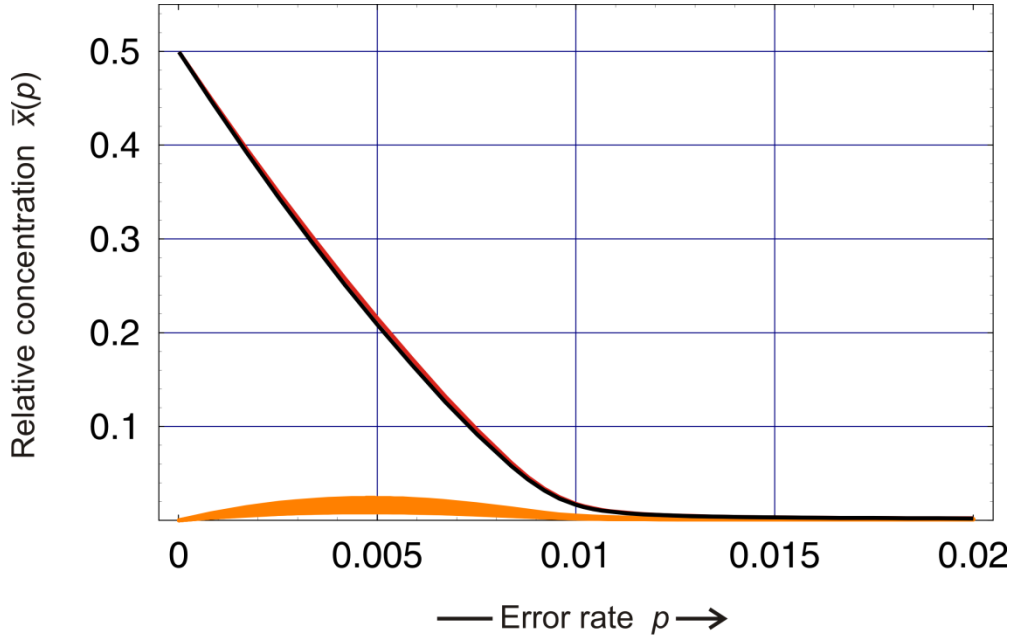
P. Schuster, J. Swetina. 1988. Bull. Math. Biol. 50:635-650




A fitness landscape including neutrality

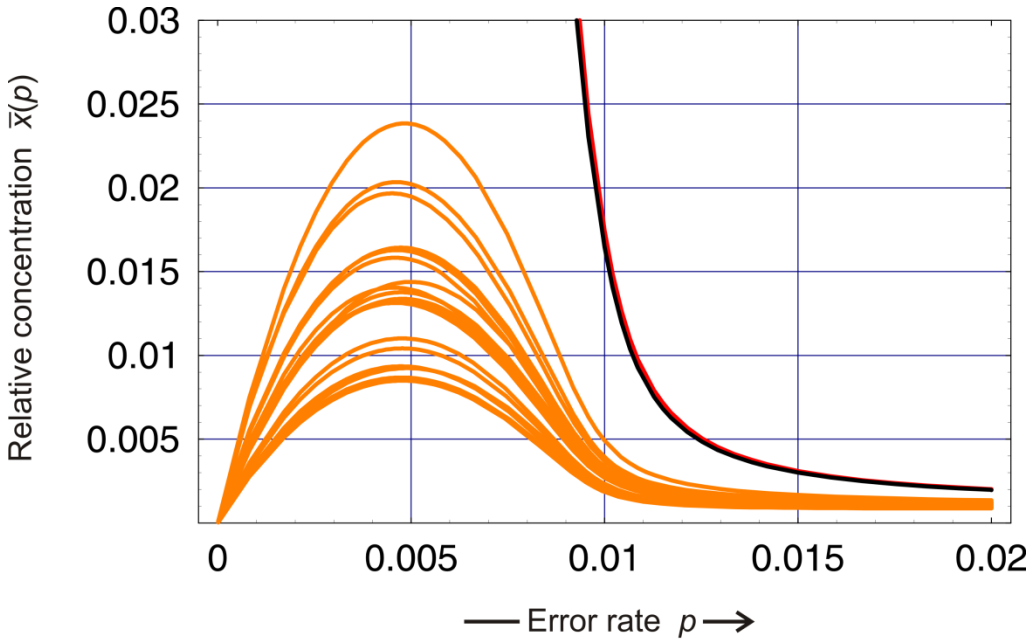

Neutral network

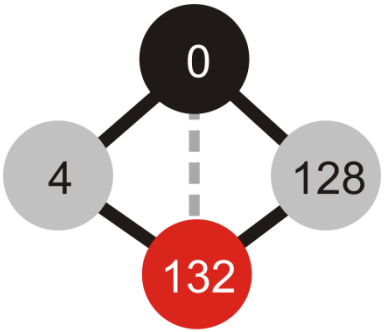
$\lambda = 0.01,\ s = 367$




Neutral network: Individual sequences

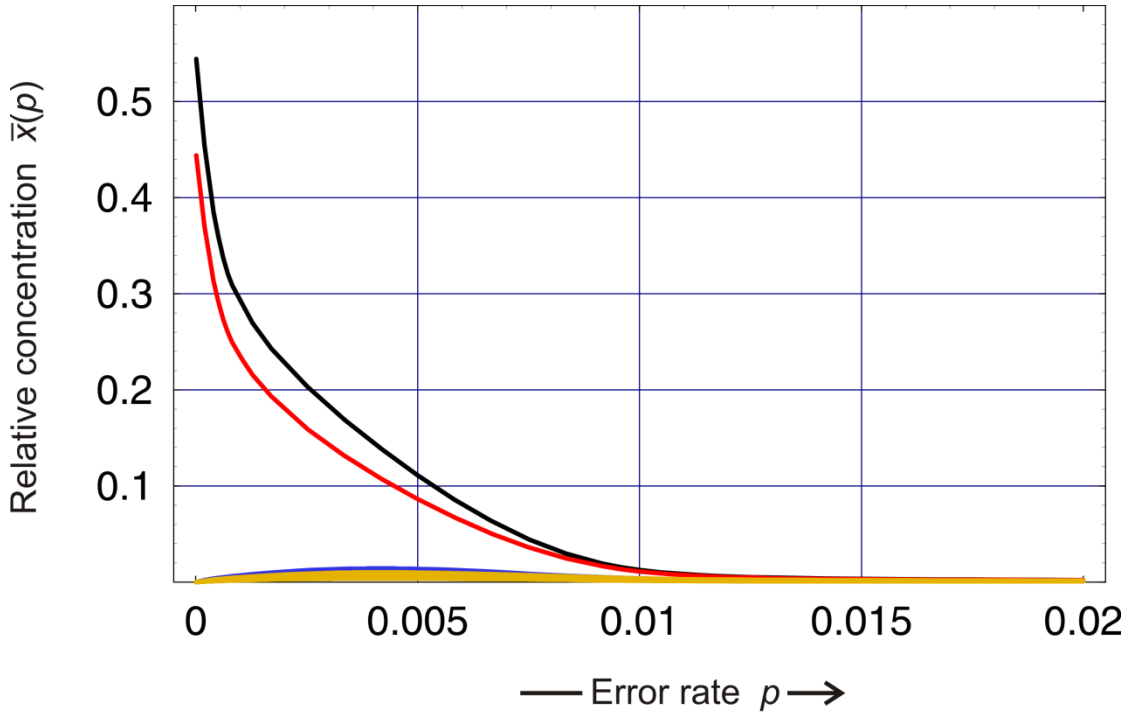
$n = 10,\ \sigma = 1.1,\ d = 1.0$





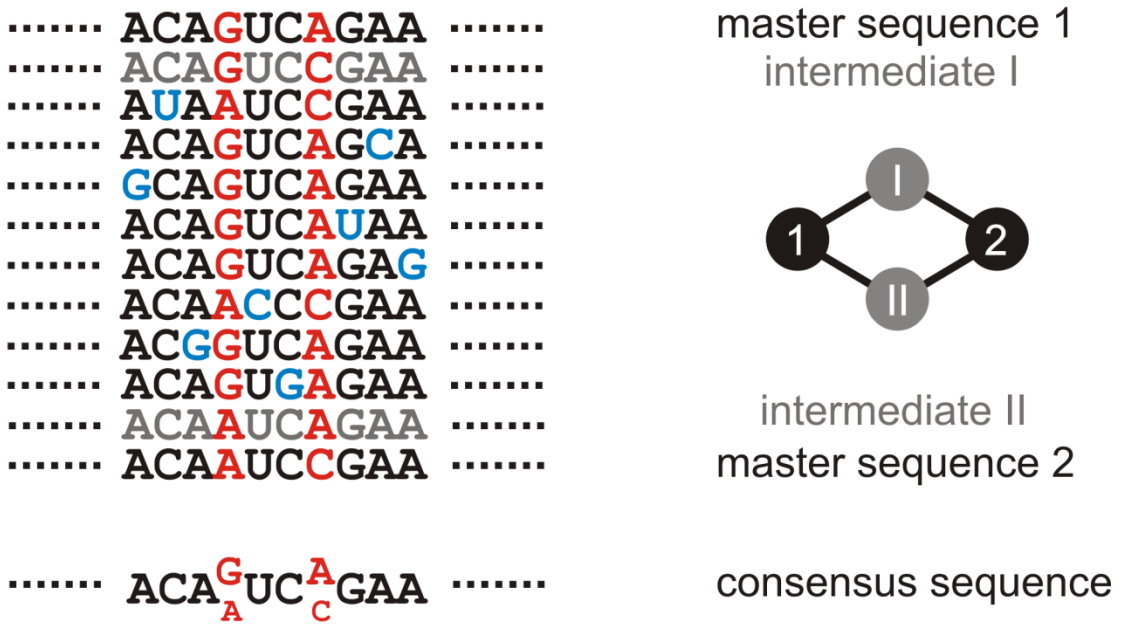

Neutral network

$\lambda = 0.01,\ s = 877$

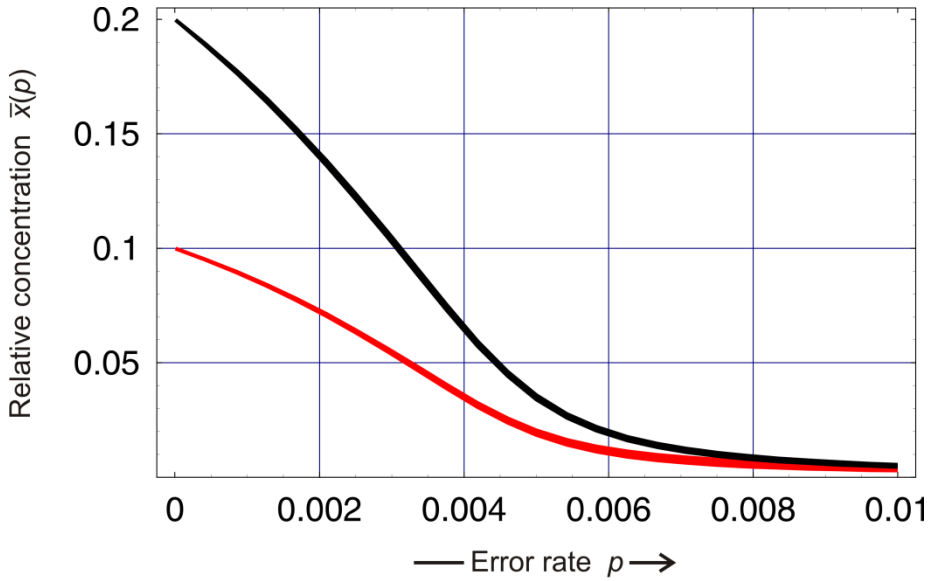



Neutral network: Individual sequences

$n = 10,\ \sigma = 1.1,\ d = 1.0$

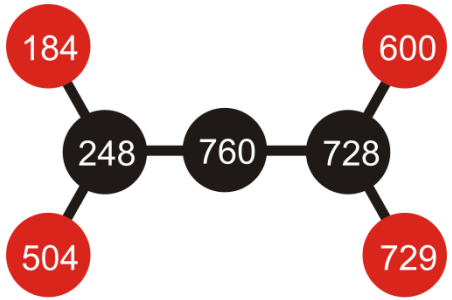




Consensus sequences of a quasispecies of two strongly coupled sequences of Hamming distance $d_H(X_i,X_j) = 1$ and 2.




Perturbation matrix W

$$\mathrm{W} = \begin{pmatrix} f & 0 & \varepsilon & 0 & 0 & 0 & 0 \\ 0 & f & \varepsilon & 0 & 0 & 0 & 0 \\ \varepsilon & \varepsilon & f & \varepsilon & 0 & 0 & 0 \\ 0 & 0 & \varepsilon & f & \varepsilon & 0 & 0 \\ 0 & 0 & 0 & \varepsilon & f & \varepsilon & \varepsilon \\ 0 & 0 & 0 & 0 & \varepsilon & f & 0 \\ 0 & 0 & 0 & 0 & \varepsilon & 0 & f \end{pmatrix}$$




Neutral network

$\lambda = 0.10$, s = 229

Adjacency matrix

Largest eigenvector of W

$$\xi_0 = (0.1, 0.1, 0.2, 0.2, 0.2, 0.1, 0.1) .$$

Neutral networks with increasing $\lambda$: $\lambda = 0.10$, s = 229

1. The origin of fitness landscape
2. Molecular biology of replication
3. Simple landscapes
4. Landscapes revisited
5. „Realistic" landscapes
6. Neutrality in evolution
7. **Perspectives**

## What remains to be done

1. How close are natural populations to a stationary solution ?

2. Upscaling to longer sequences

3. Extension to the AUGC alphabet

4. Stochasticity described by chemical master equations or birth-and death processes

5. Discrete versions of the model for synchronized generations

# Exploration of realistic fitness landscapes

1. **High dimensionality**, which is hard to visualize.

2. **Ruggedness**: nearby lying mutations may lead to very large effects or no effects at all.

3. **Neutrality**: there is always a non-negligible fraction of mutations that cannot be distinguished by selection.

4. **High efficiency sequencing** and **high-throughput screening** methods will allow for fast harvesting of large amounts of data.

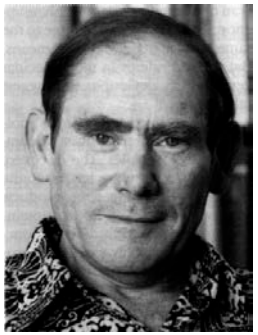
5. **New theoretical approaches** will be used to reduce the amount of data required for a understanding of evolutionary dynamics.

# Advantages of the molecular approach

1. Complex reproduction mechanisms are readily included.

2. Gene regulation – DNA or RNA based – is chemical kinetics!

3. Accounting for epigenetic effects requires just the simultaneous consideration of several generations.



Sydney Brenner, 1927 -

What else is epigenetics than a funny form of enzymology ? Each protein, after all, comes of some piece of DNA.

# Coworkers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Universität Wien

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