Theorie und Modellierung der Molekularen Evolution

Peter Schuster

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Darwin-Tag der Bayerischen Akademie der Wissenschaften

München, 12.02.2009

Web-Page for further information:

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

1. Charles Darwin heute
2. Darwins Prinzip der natürlichen Auslese
3. Vermehrung von Molekülen
4. Chemische Kinetik der molekularen Evolution
5. Evolutionsexperimente mit Molekülen
6. Simulation der Optimierung von Strukturen
7. Ursachen und Konsequenzen der Neutralität

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Kardinal Christoph Schönborn, ***Finding Design in Nature***, commentary in *The New York Times*, July 5, 2005

„ ... Evolution in the sense of common ancestry might be true, but evolution in the Neo-Darwinian sense – an unguided, unplanned process of random variation and natural selection – is not. Any system of thought that denies or seeks to explain away the overwhelming evidence for design in biology is ideology, not science.

... Scientific theories that try to explain away the appearance of design as the result of ‚chance and necessity' are not scientific at all, but ... an abdication of human intelligence."

Peter Schuster. ***Evolution and design. The Darwinian theory of evolution is a scientific fact and not an ideology.*** Complexity **11**(1):12-15, 2006

Peter Schuster. ***Evolution und Design. Versuch einer Bestandsaufnahme der Evolutionstheorie***.

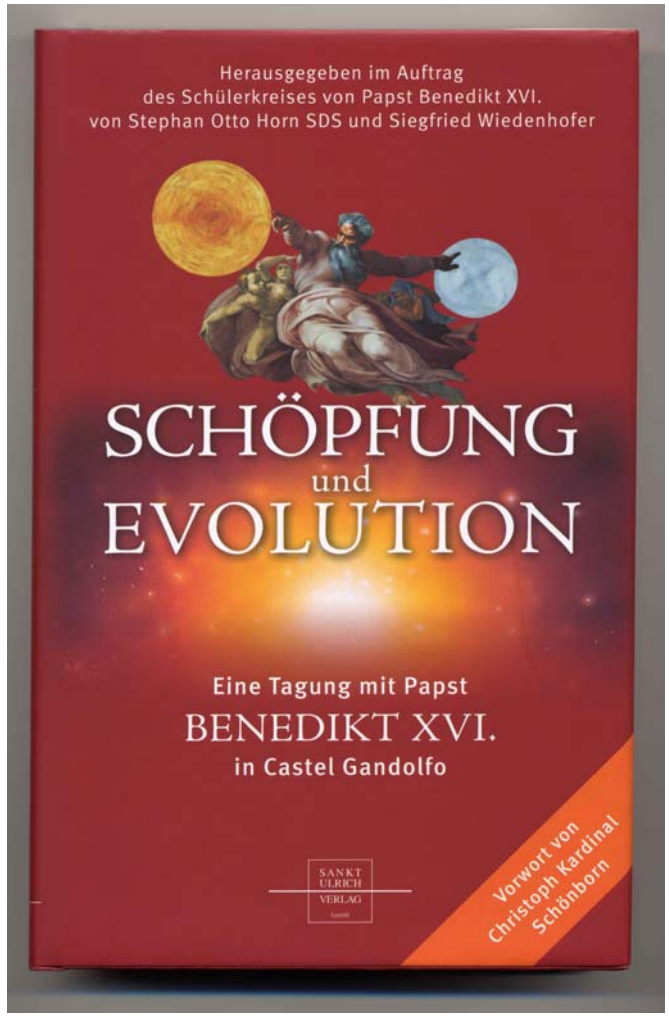
In: Stephan Otto Horn und Siegfried Wiedenhofer, Eds.

Schöpfung und Evolution. Eine Tagung mit Papst Benedikt XVI
in Castel Gandolfo. Sankt Ulrich Verlag, Augsburg 2007, pp.25-56.

English translation:

Creation and Evolution.

Ignatius Press, San Francisco, CA, 2008



„You care for nothing but shooting, dogs and rat-catching“, Robert Darwin told his son, „and you will be a disgrace to yourself and all your family“. Yet the feckless boy is everywhere. Charles Darwin gets so much credit, we can‘t distinguish evolution from him.

Carl Safina. ***Darwinism must die so that evolution may live***.

The New York Times, February 12, 2009

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Equating evolution with Charles Darwin ignores 150 years of discoveries, including most of what scientists understand about evolution. Such as Gregor Mendel‘s pattern of heredity (which gave Darwin‘s idea of natural selection a mechanism – genetics – by which it could work), the discovery of DNA (which gave genetics a mechanism and let us see evolutionary lineages), developmental biology (which gives DNA a mechanism), studies documenting evolution in nature (which converted the hypothetical to observable fact), evolution‘s role in medicine and disease (bringing immediate relevance to the topic), and more.

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By propounding „Darwinism“, even scientists and science writers perpetuate an impression that evolution is about one man, one book, one „theory“. The ninth-century Buddhist master Lin Chi said, „If you meet the Buddha on the road, kill him.“ The point is that making a master teacher into a sacred fetish misses the essence of his teaching. So let us now kill Darwin.

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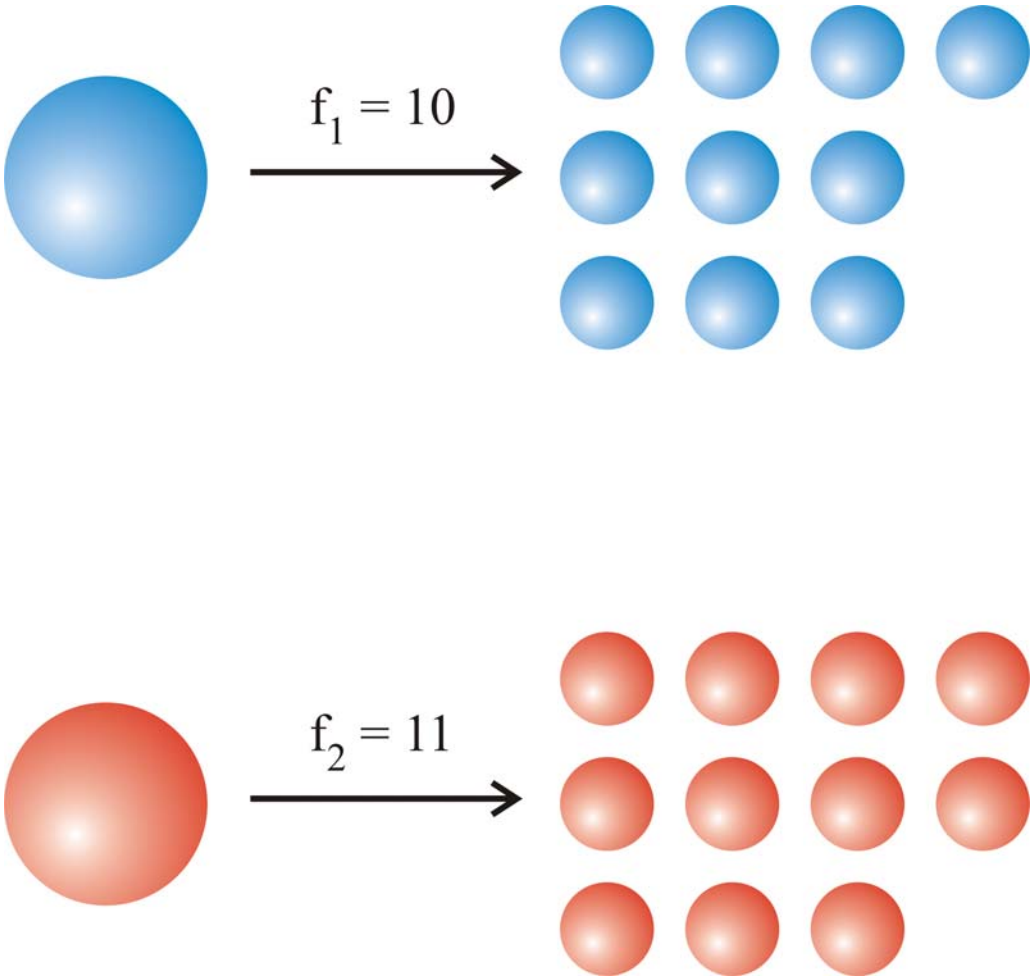
Drei notwendige Bedingungen für Darwinsche Evolution:

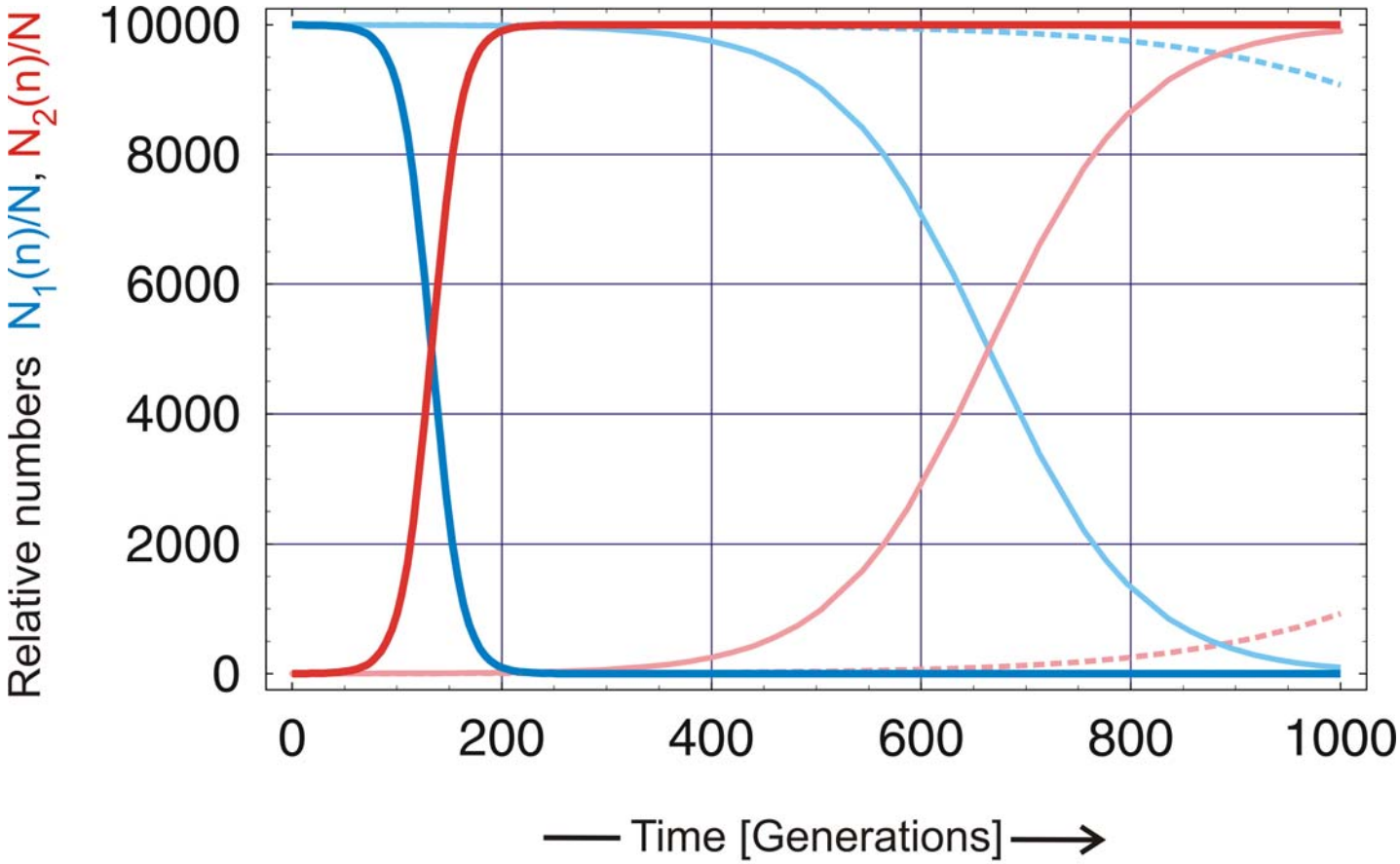
1. **Vermehrung**
2. **Variation**
3. **Selektion**

Empirisch erkanntes Prinzip der natürlichen Auslese



$$s = \frac{f_2 - f_1}{f_1} = 0.1$$

Two variants with a mean progeny of ten or eleven descendants



$$N_1(0) = 9999,\ N_2(0) = 1; \quad s = 0.1, 0.02, 0.01$$

Selection of advantageous mutants in populations of N = 10 000 individuals

Genotype, Genom

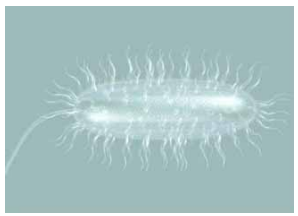
GCGGATTTAGCTCAGTTGGGAGAGCGCCAGACTGAAGATCTGGAGGTCCTGTGTTCGATCCACAGAATTCGCACCA

Biochemie
Strukturbiologie
Molekularbiologie
Molekulare Evolution
Molekulargenetik
Systembiologie
Bioinfomatik

Genetik
Epigenetik
Umwelt

Entwicklung

Zellbiologie
Entwicklungsbiologie
Neurobiologie
Mikrobiologie
Botanik und Zoologie
Anthropologie
Ökologie



Phänotyp

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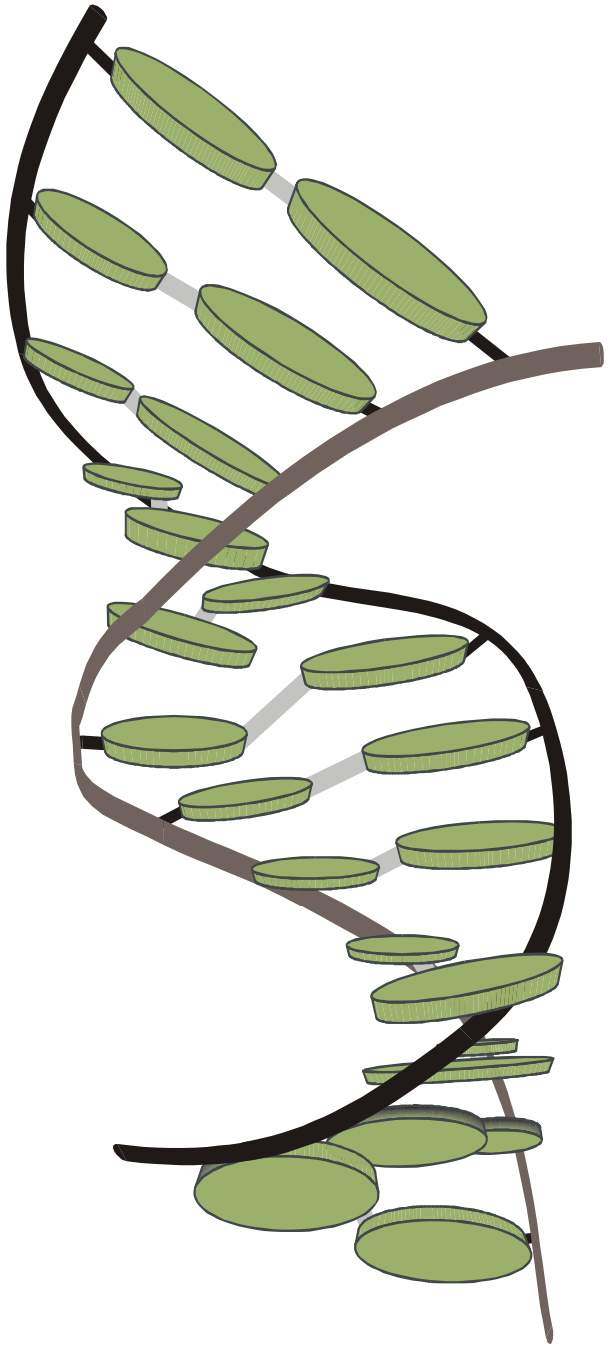
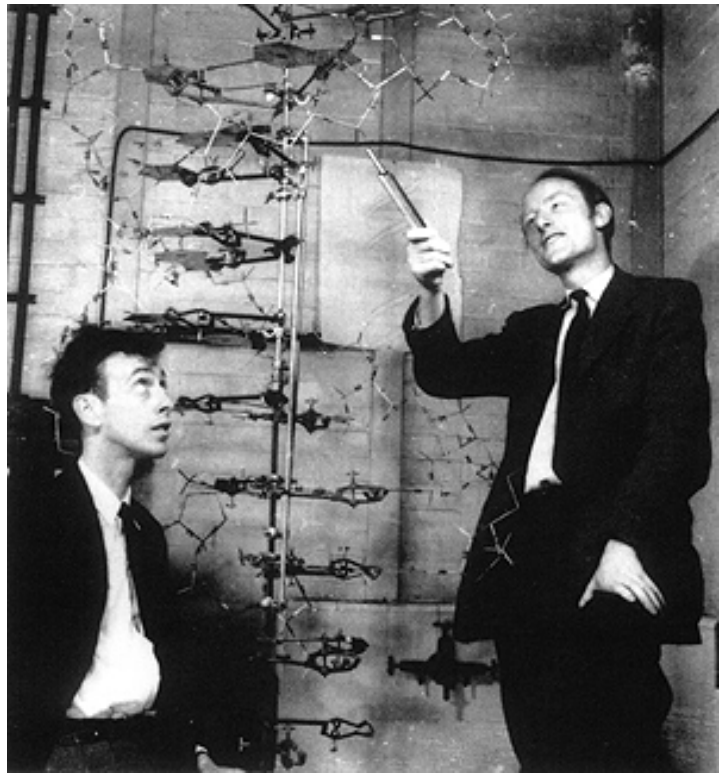


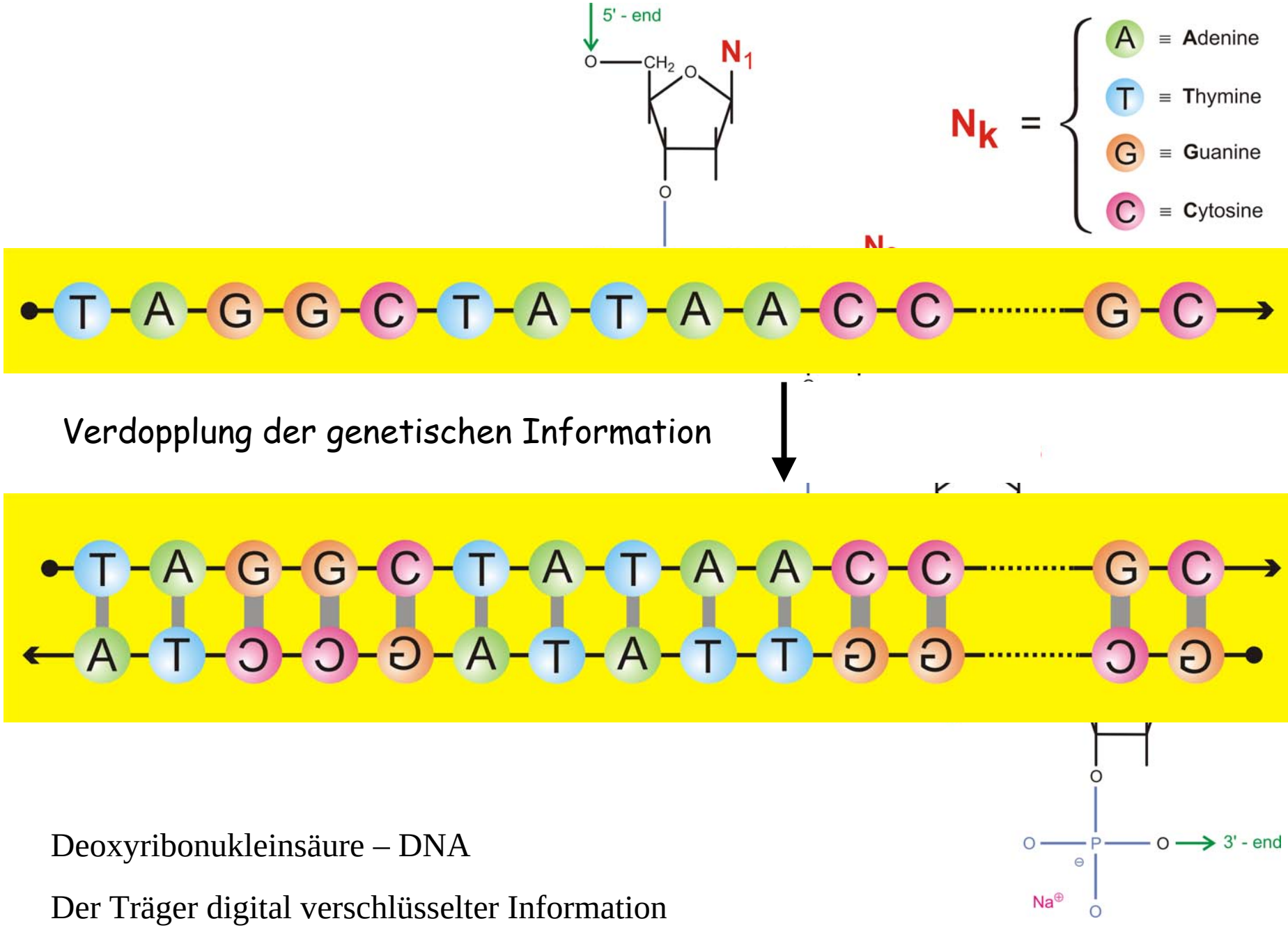


James D. Watson, 1928-, and Francis H.C. Crick, 1916-2004

Nobel prize 1962

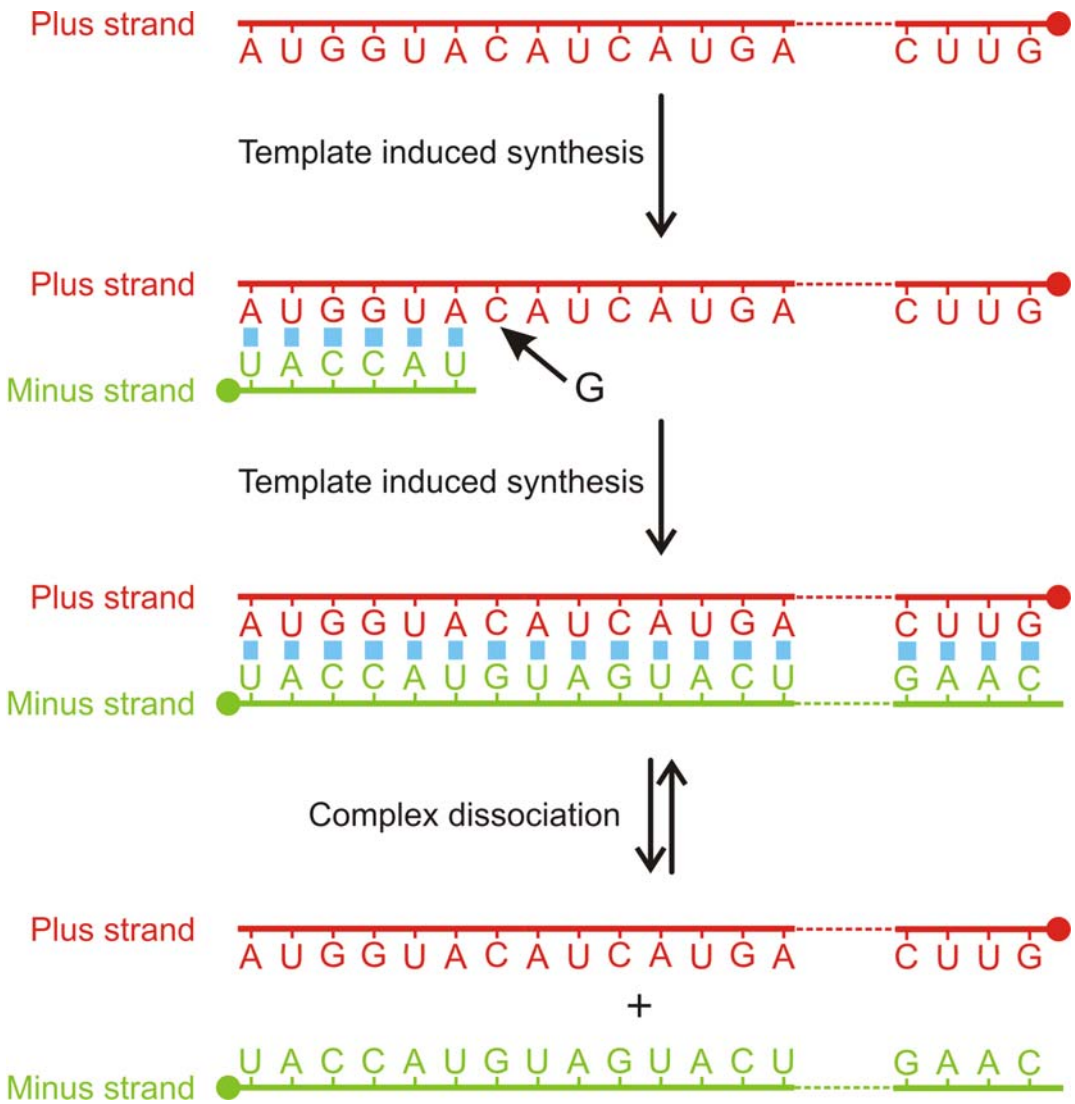
1953 – 2003 fifty years double helix

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



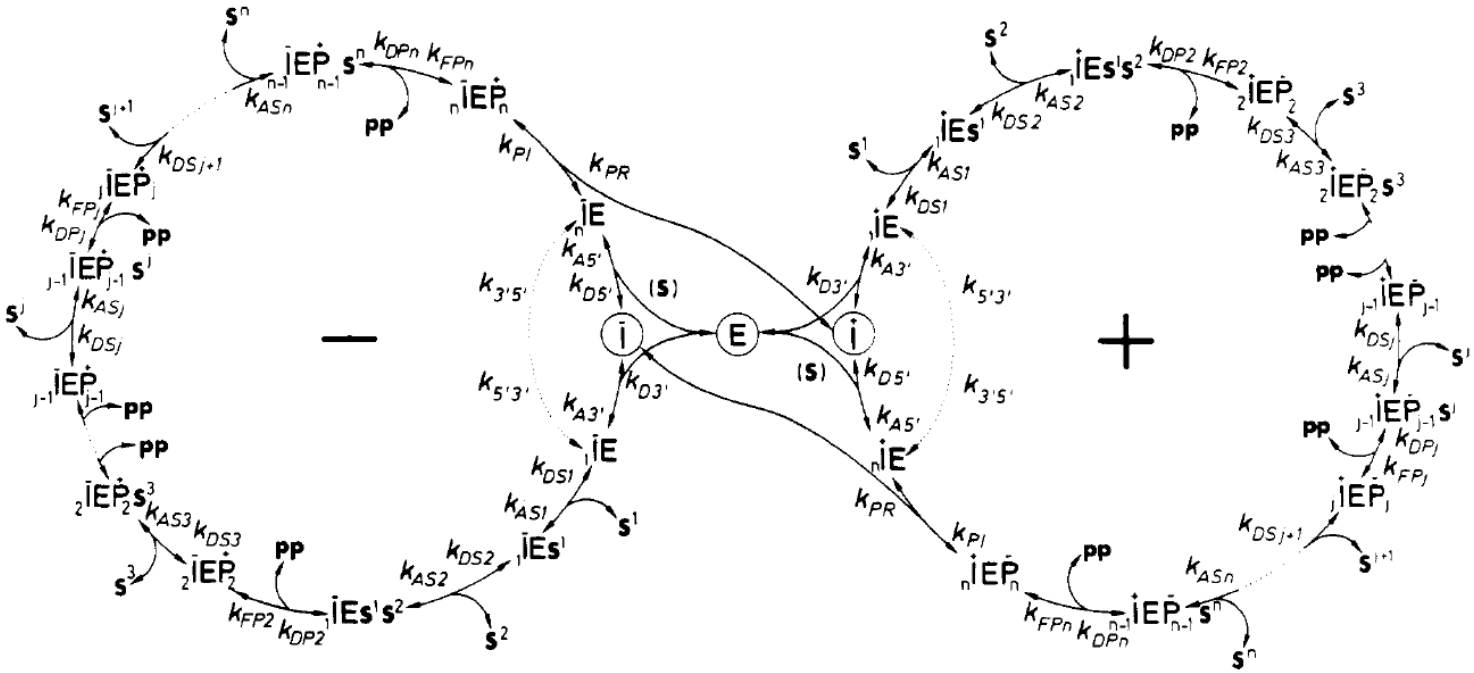
Deoxyribonukleinsäure – DNA

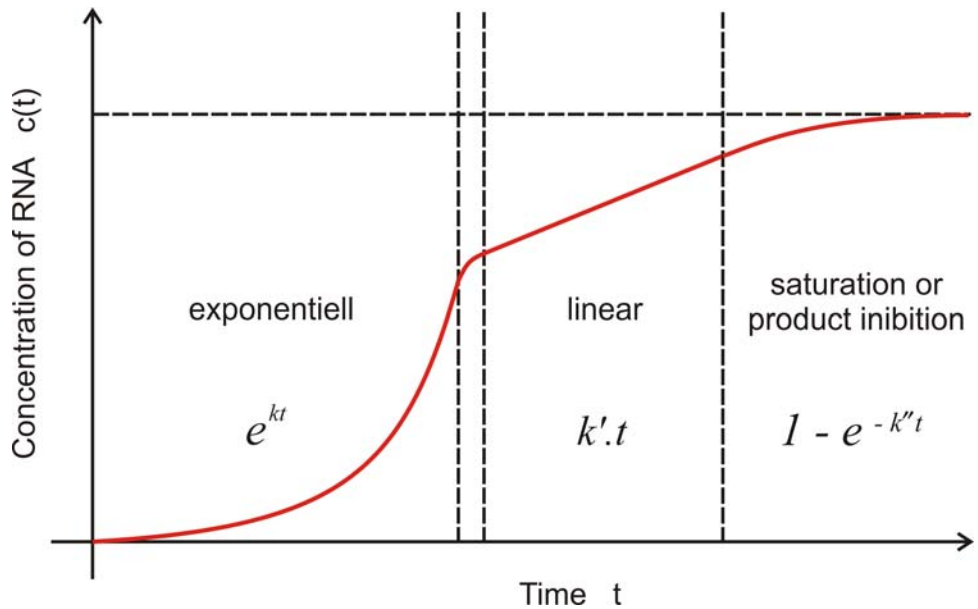
Der Träger digital verschlüsselter Information



Complementary replication is the simplest copying mechanism of RNA.
Complementarity is determined by Watson-Crick base pairs:

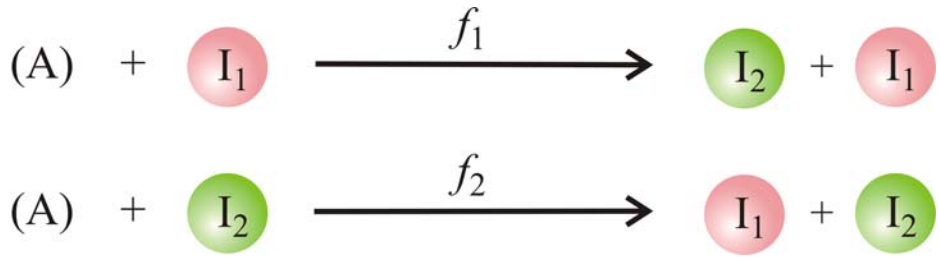
G≡C and **A=U**





Kinetics of RNA replication

C.K. Biebricher, M. Eigen, W.C. Gardiner, Jr.
Biochemistry **22**:2544-2559, 1983



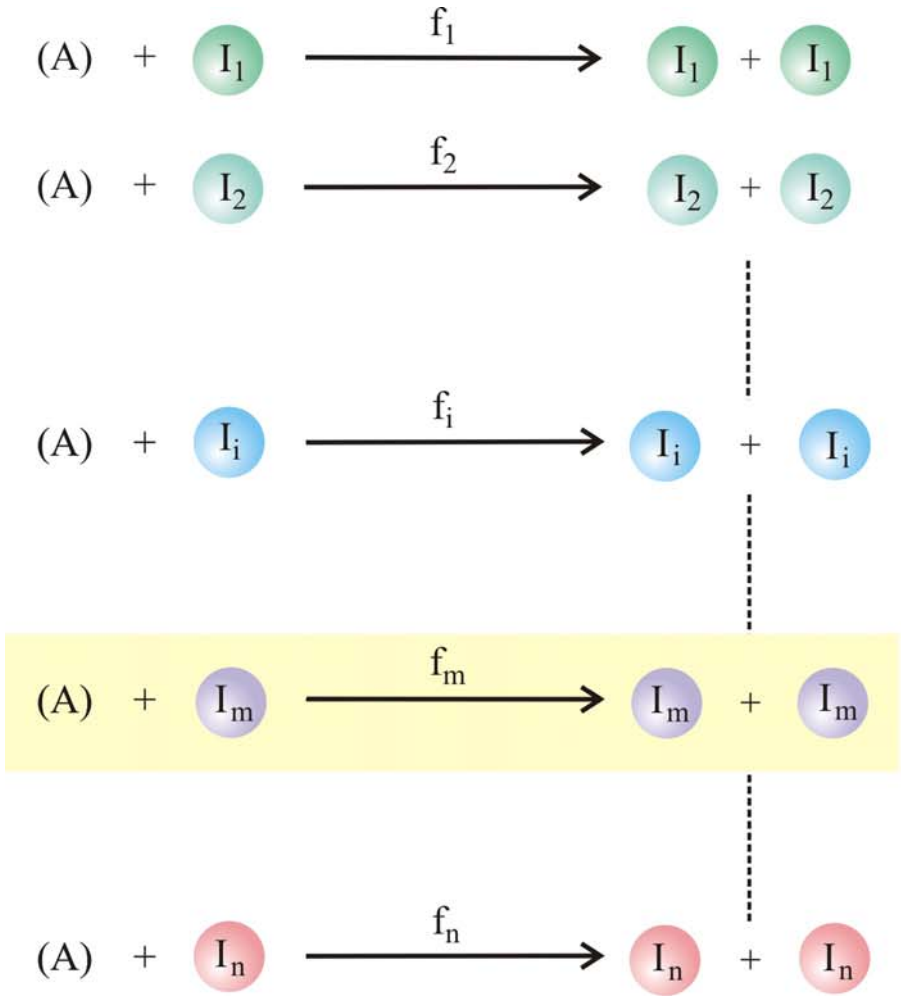
$$\frac{dx_1}{dt} = f_2\, x_2 \quad \text{and} \quad \frac{dx_2}{dt} = f_1\, x_1$$

$$x_1 = \sqrt{f_2}\;\xi_1\,, \quad x_2 = \sqrt{f_1}\;\xi_2\,, \quad \zeta = \xi_1 + \xi_2\,, \quad \eta = \xi_1 - \xi_2\,, \quad f = \sqrt{f_1 f_2}$$

$$\eta(t) = \eta(0)\, e^{-ft}$$

$$\zeta(t) = \zeta(0)\, e^{ft}$$

Complementary replication as the simplest molecular mechanism of reproduction



$$dx_i / dt = f_i x_i - x_i \Phi = x_i (f_i - \Phi)$$

$$\Phi = \Sigma_j f_j x_j ; \quad \Sigma_j x_j = 1 ; \quad i,j = 1,2,...,n$$

$$[I_i] = x_i \geq 0 ; \quad i = 1,2,...,n ;$$

$$[A] = a = \text{constant}$$

$$f_m = \max \{f_j; j=1,2,...,n\}$$

$$x_m(t) \rightarrow 1 \quad \text{for} \quad t \rightarrow \infty$$

Selection in an ensemble of replicating molecules

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Drei notwendige Bedingungen für Darwinsche Evolution:

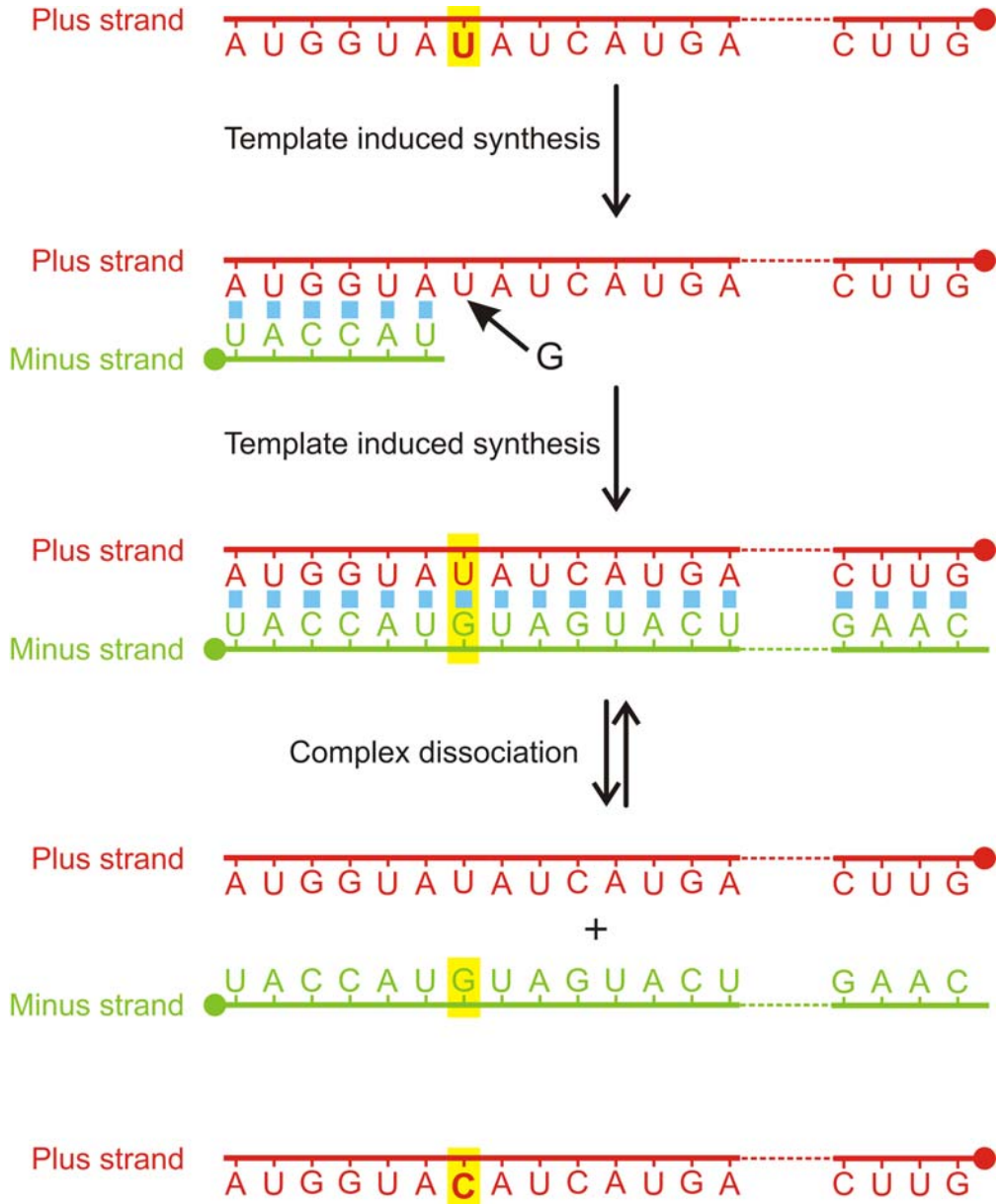
1. **Vermehrung,**
2. **Variation**, and
3. **Selektion**.

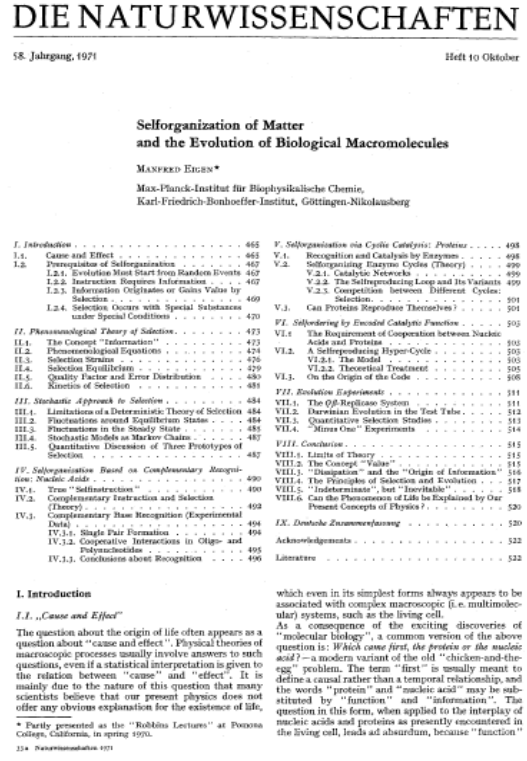
Variation in Form von Rekombination und/oder Mutation verändert die **Genotypen** wogegen **Selektion** nur auf den **Phänotypen** operiert.

Im Darwinschen Szenario treten **Variationen** in Form von Rekombinations- und/oder Mutationsereignissen **unkorreliert** mit ihren **Effekt auf den Selektionprocess** auf und erscheinen daher **zufällig**.

Alle drei Bedingungen werden nicht nur von zellulären Organismen erfüllt sondern auch von **Molekülen** in geigneten **zellfreien Assays**.

Mutation as an error
in replication





DIE NATURWISSENSCHAFTEN

58. Jahrgang, 1971 — Heft 10 Oktober

Selforganization of Matter and the Evolution of Biological Macromolecules

MANFRED EIGEN*

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

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

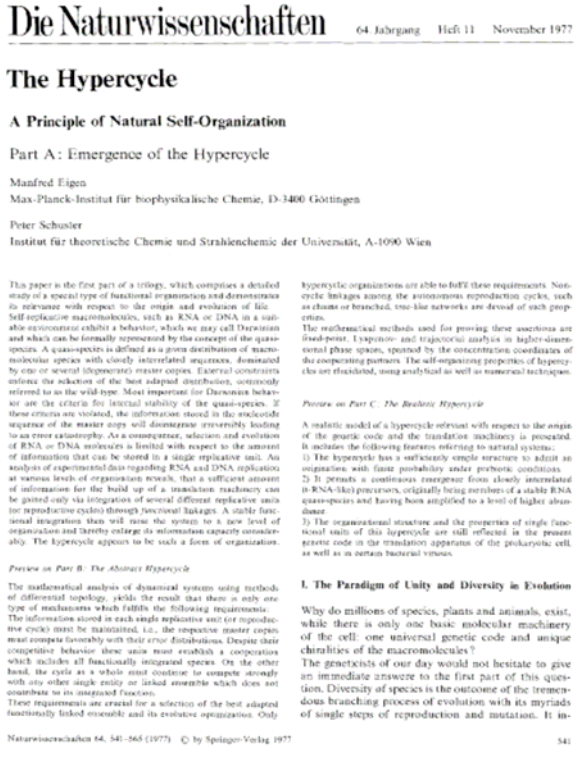
1. Introduction

1.1. „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-the-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"

1971



Die Naturwissenschaften 64. Jahrgang Heft 11 November 1977

The Hypercycle

A Principle of Natural Self-Organization

Part A: Emergence of the Hypercycle

Manfred Eigen
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Peter Schuster
Institut für theoretische Chemie und Strahlenchemie der Universität, A-1090 Wien

This paper is the first part of a trilogy, which comprises a detailed study of a special type of functional organization and demonstrates its relevance with respect to the origin and evolution of life. Self-replicative macromolecules, such as RNA or DNA in a suitable environment exhibit a behavior, which we may call Darwinian and which can be formally represented by the concept of the quasi-species. A quasi-species is defined as a given distribution of macromolecular species with closely interrelated sequences, dominated by one or several (degenerate) master copies. External constraints enforce the selection of the best adapted distribution, commonly referred to as the wild-type. Most important for Darwinian behavior are the criteria for internal stability of the quasi-species. If these criteria are violated, the information stored in the nucleotide sequence of the master copy will deteriorate irreversibly leading to an error catastrophy. As a consequence, selection and evolution of RNA or DNA molecules is limited with respect to the amount of information that can be stored in a single replicative unit. An analysis of experimental data regarding RNA and DNA replication at various levels of organization reveals, that a sufficient amount of information for the build up of a translation machinery can be gained only via integration of several different replicative units (or reproductive cycles) through functional linkages. A stable functional integration then will raise the system to a new level of organization and thereby enlarge its information capacity considerably. The hypercycle appears to be such a form of organization.

Preview on Part B: The Abstract Hypercycle

The mathematical analysis of dynamical systems using methods of differential topology, yields the result that there is only one type of mechanisms which fulfills the following requirements: The information stored in each single replicative unit (or reproductive cycle) must be maintained, i.e., the respective master copies must compete favorably with their error distributions. Despite their competitive behavior these units must establish a cooperation which includes all functionally integrated species. On the other hand, the cycle as a whole must continue to compete strongly with any other single entity or linked ensemble which does not contribute to its integrated function.
These requirements are crucial for a selection of the best adapted functionally linked ensemble and its evolutive optimization. Only hypercyclic organizations are able to fulfil these requirements. Non-cycle linkages among the autonomous reproduction cycles, such as chains or branched, tree-like networks are devoid of such properties.
The mathematical methods used for proving these assertions are fixed-point, Lyapunov- and trajectorial analysis in higher-dimensional phase spaces, spanned by the concentration coordinates of the cooperating partners. The self-organizing properties of hypercycles are elucidated, using analytical as well as numerical techniques.

Preview on Part C: The Realistic Hypercycle

A realistic model of a hypercycle relevant with respect to the origin of the genetic code and the translation machinery is presented. It includes the following features referring to natural systems:
1) The hypercycle has a sufficiently simple structure to admit an origination with finite probability under prebiotic conditions.
2) It permits a continuous emergence from closely interrelated (t-RNA-like) precursors, originally being members of a stable RNA quasi-species and having been amplified to a level of higher abundance.
3) The organizational structure and the properties of single functional units of this hypercycle are still reflected in the present genetic code in the translation apparatus of the prokaryotic cell, as well as in certain bacterial viruses.

I. The Paradigm of Unity and Diversity in Evolution

Why do millions of species, plants and animals, exist, while there is only one basic molecular machinery of the cell: one universal genetic code and unique chiralities of the macromolecules?
The geneticists of our day would not hesitate to give an immediate answere to the first part of this question. Diversity of species is the outcome of the tremendous branching process of evolution with its myriads of single steps of reproduction and mutation. It in-

1977

Molecular Quasi-Species†

Manfred Eigen,* John McCaskill,
Max Planck Institut für biophysikalische Chemie, Am Fassberg, D 3400 Göttingen-Nikolausberg, BRD
and Peter Schuster*
Institut für theoretische Chemie und Strahlenchemie, der Universität Wien, Währinger Strasse 17, A-1090 Wien, Austria (Received: June 9, 1988)

The molecular quasi-species model describes the physicochemical organization of monomers into an ensemble of heteropolymers with combinatorial complexity by ongoing template polymerization. Polynucleotides belong to the simplest class of such molecules. The quasi-species itself represents the stationary distribution of macromolecular sequences maintained by chemical reactions effecting error-prone replication and by transport processes. It is obtained deterministically, by mass-action kinetics, as the dominant eigenvalue of a *value* matrix, **W**, which is derived directly from chemical rate coefficients, but it also exhibits stochastic features, being composed to a significant fraction of unique individual macromolecular sequences. The quasi-species model demonstrates how macromolecular information originates through specific nonequilibrium autocatalytic reactions and thus forms a bridge between reaction kinetics and molecular evolution. Selection and evolutionary optimization appear as new features in physical chemistry. Concentration bias in the production of mutants is a new concept in population genetics, relevant to frequently mutating populations, which is shown to greatly enhance the optimization properties. The present theory relates to asexually replicating ensembles, but this restriction is not essential. A sharp transition is exhibited between a drifting population of essentially random macromolecular sequences and a localized population of close relatives. This transition at a threshold error rate was found to depend on sequence lengths, distributions of selective values, and population sizes. It has been determined generically for complex landscapes and for special cases, and, it was shown to persist generically in the presence of nearly neutral mutants. Replication dynamics has much in common with the equilibrium statistics of complex spin systems: the error threshold is equivalent to a magnetic order-disorder transition. A rational function of the replication accuracy plays the role of temperature. Experimental data obtained from test-tube evolution of polynucleotides and from studies of natural virus populations support the quasi-species model. The error threshold seems to set a limit to the genome lengths of several classes of RNA viruses. In addition, the results are relevant even in eucaryotes where they contribute to the exon-intron debate.

1. Molecular Selection

Our knowledge of physical and chemical systems is, in a final analysis, based on models derived from repeatable experiments. While none of the classic and rather besieged list of properties rounded up to support the intuition of a distinction between the living and nonliving—metabolism, self-reproduction, irritability, and adaptability, for example—intrinsically limit the application of the scientific method, a determining role by unique or *individual* entities comes into conflict with the requirement of repeatability. Combinatorial variety, such as that in heteropolymers based on even very small numbers of different bases, even just two, readily provides numbers of different entities so enormous that neither consecutive nor parallel physical realization is possible. The physical chemistry of finite systems of such macromolecules must deal with both known regularities and the advent of unique copolymeric sequences. Normally this would present no difficulty in a statistical mechanical analysis of typical behavior, where rare events play no significant role, but with autocatalytic polymerization processes even unique single molecules may be amplified to determine the fate of the entire system. Potentially creative, self-organizing around unique events, the dynamics of this simplest living chemical system is invested with regularities that both allow and limit efficient adaptation. The quasi-species model is a study of these regularities.

The fundamental regularity in living organisms that has invited explanation is adaptation. Why are organisms so well fitted to their environments? At a more chemical level, why are enzymes optimal catalysts? Darwin's theory of natural selection has provided biologists with a framework for the answer to this question. The present model is constructed along Darwinian lines but in terms of specific macromolecules, chemical reactions, and physical processes that make the notion of survival of the fittest precise. Not only does the model give an understanding of the physical limitations of adaptation, but also it provides new insight into the role of chance in the process. For an understanding of the structure of this minimal chemical model it is first necessary to recall the conceptual basis of Darwin's theory.

Darwin recognized that new inheritable adaptive properties were not induced by the environment but arose independently in the production of offspring. Lasting adaptive changes in a population could only come about by natural selection of the heritable traits or *genotype* based on the full characteristics or *phenotype* relevant for producing offspring. A process of chance, i.e., uncorrelated with the developed phenotype, controls changes in the genotype from one generation to the next and generates the diversity necessary for selection. Three factors have probably prevented chemists from gaining a clear insight into these phenomena in the past, despite the discovery of the polymeric nature of the genotype (DNA): the complexity of a minimum replication phenotype, the problem of dealing with a huge number of variants, and the nonequilibrium nature of these ongoing processes.

The formulation of a tractable chemical model based on Darwin's principle may be understood in several steps:

1. The major constituents of the system have to be inherently self-reproductive. Only two classes of molecules are presently

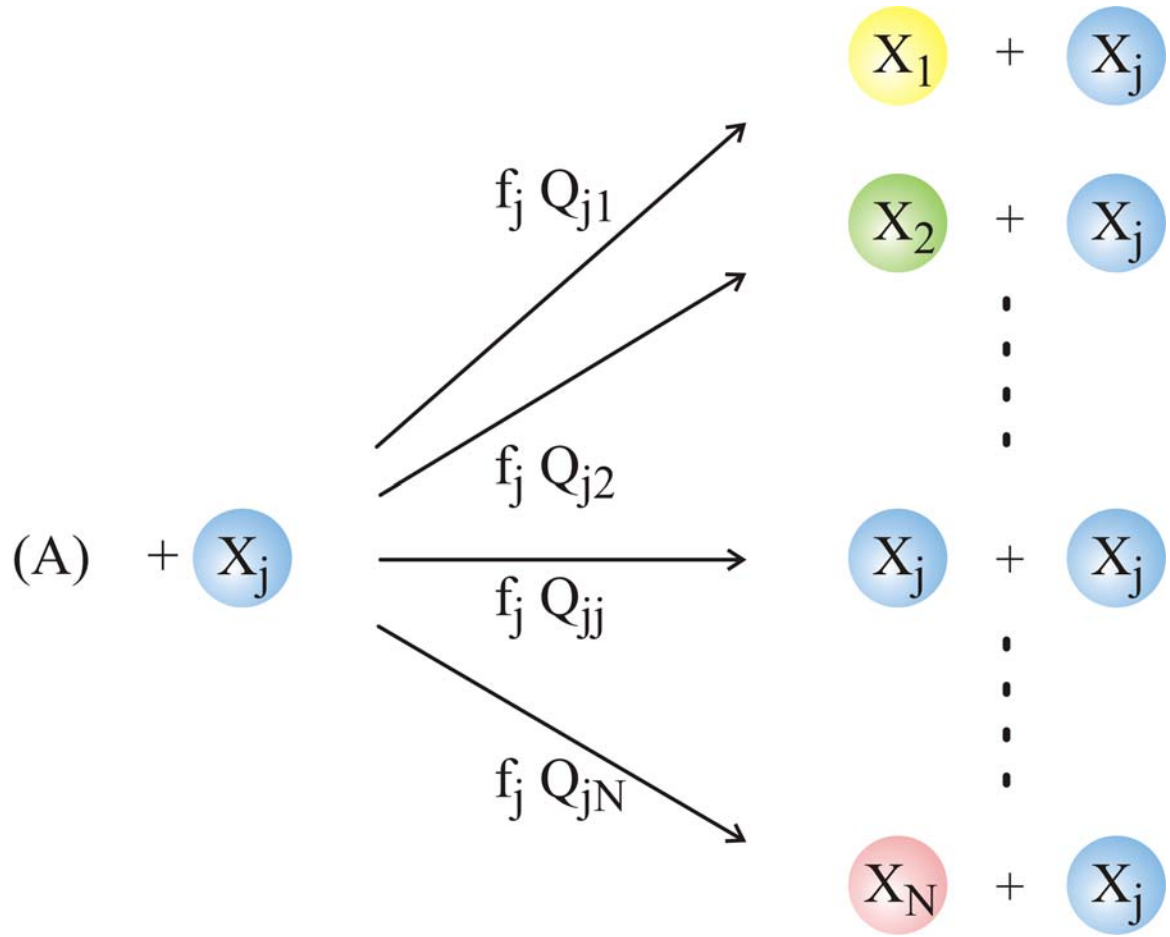
† This is an abridged account of the quasi-species theory that has been submitted in comprehensive form to *Advances in Chemical Physics*.[1]

(1) Eigen, M.; McCaskill, J. S.; Schuster, P. *Adv. Chem. Phys.*, in press.

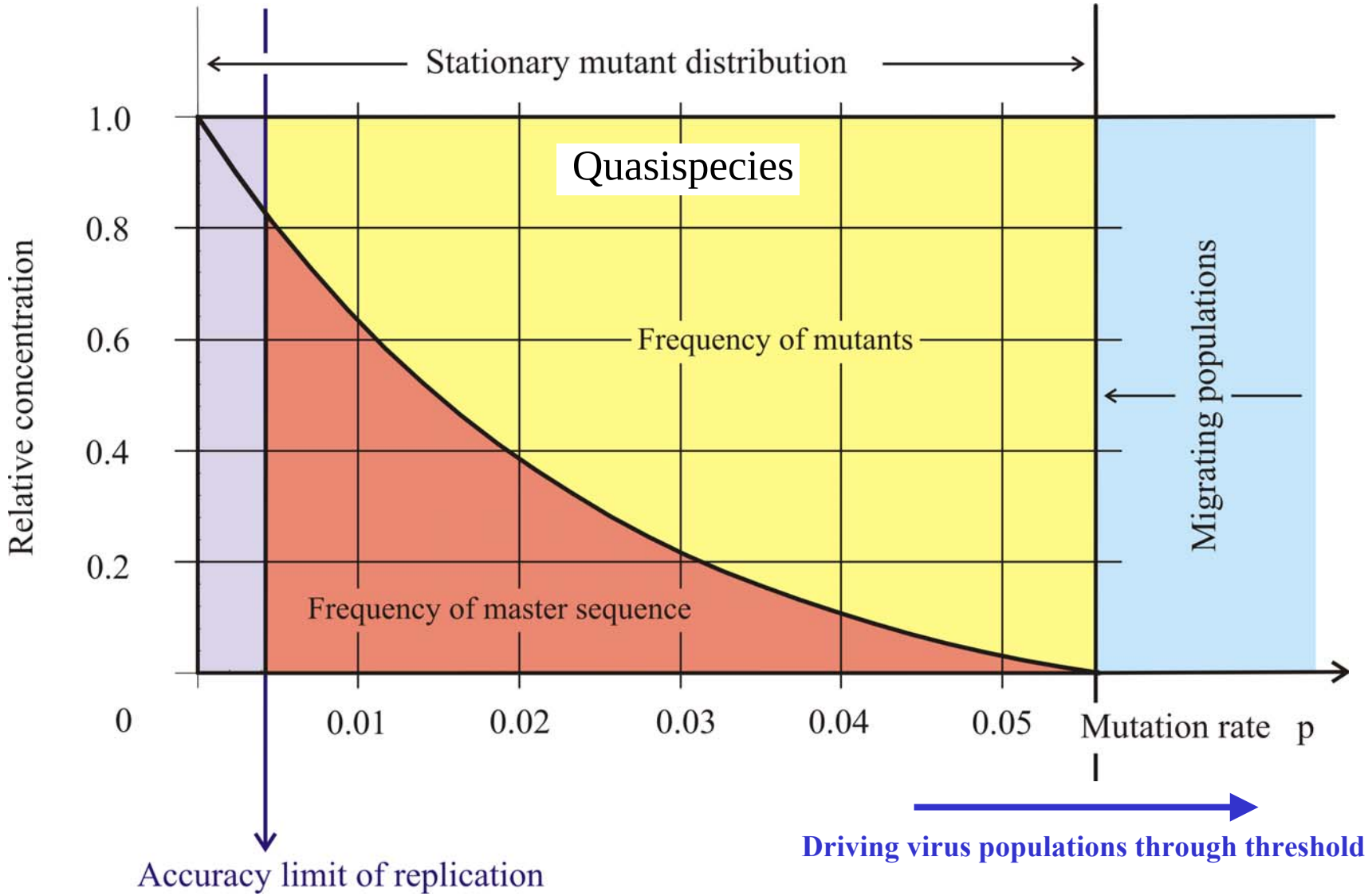
0022-3654/88/2092-6881$01.50/0

1988

Chemical kinetics of molecular evolution

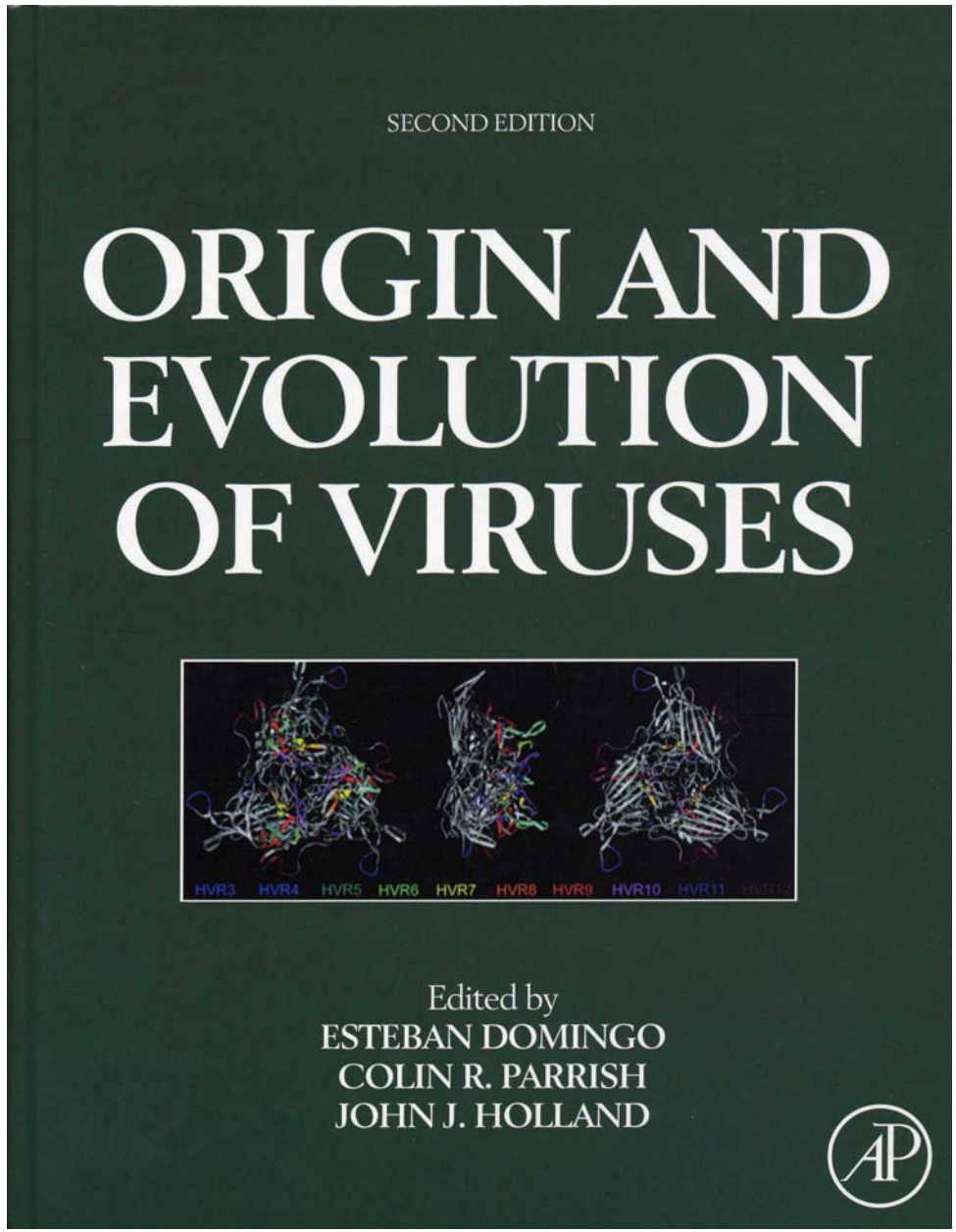


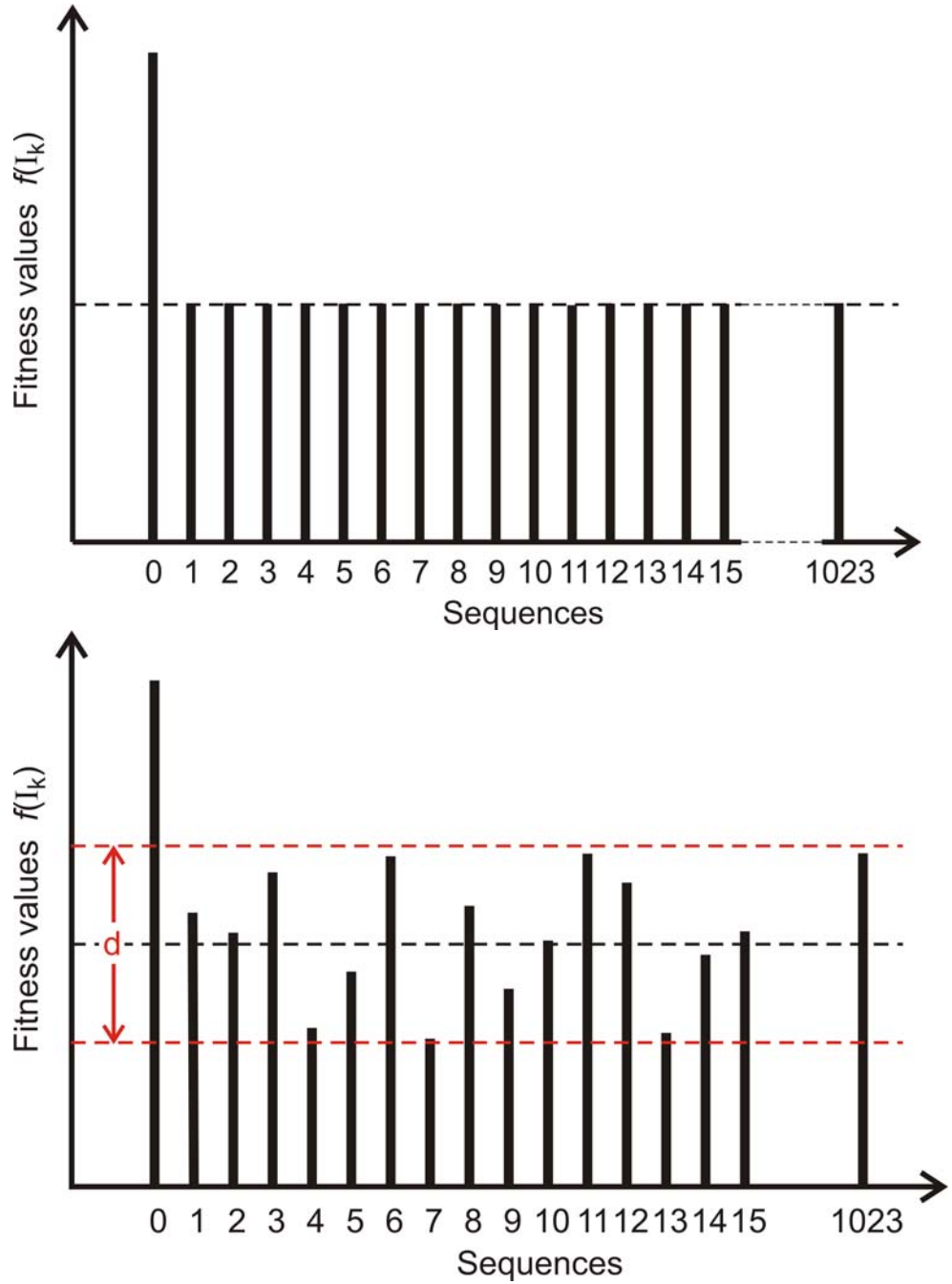
Chemical kinetics of replication and mutation as parallel reactions



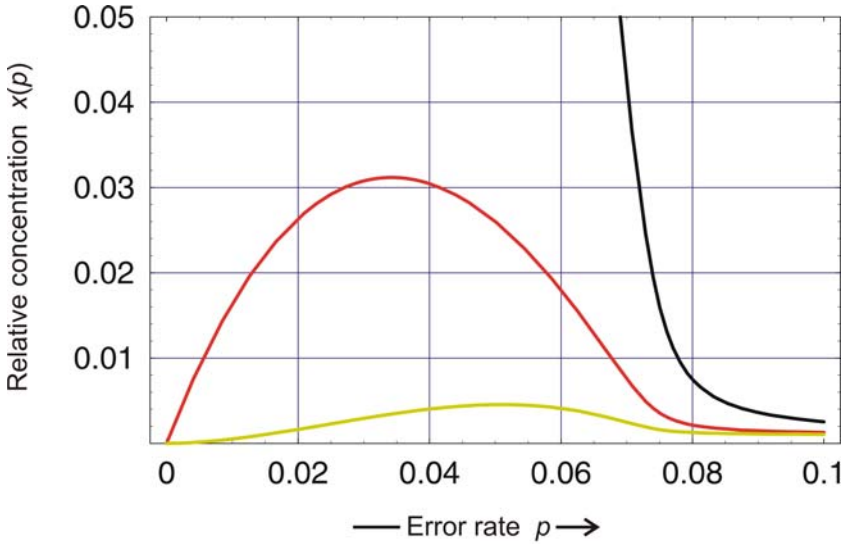
The error threshold in replication-mutation ensembles

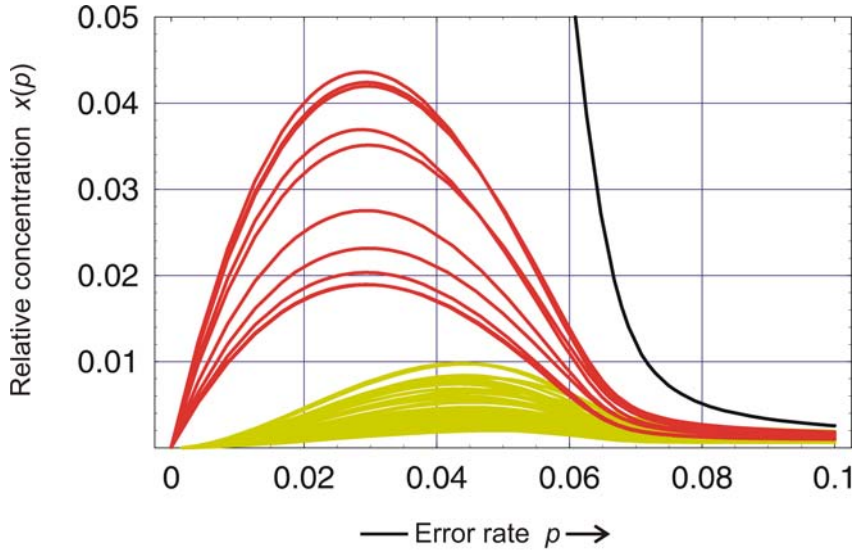
Molecular evolution of viruses

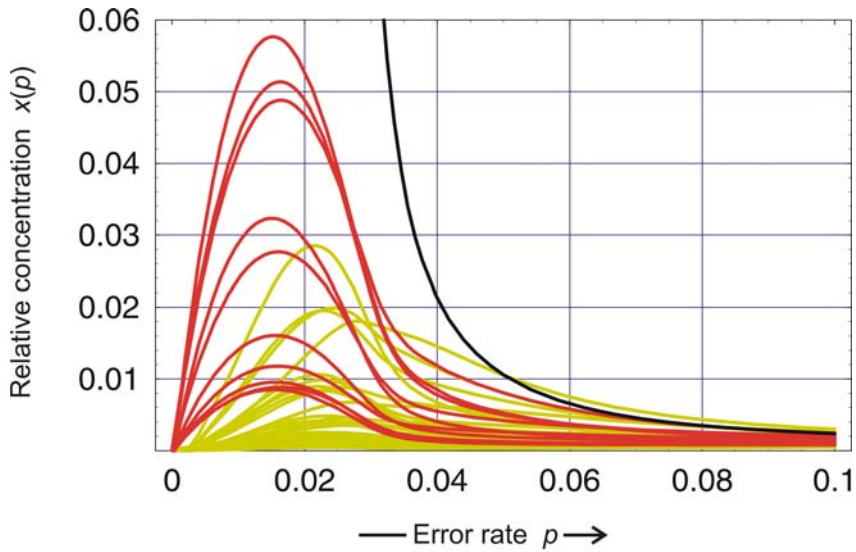




Fitness landscapes showing error thresholds







Error threshold: Individual sequences

n = 10, σ = 2 and d = 0, 1.0, 1.85

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Evolution of RNA molecules based on Qβ phage

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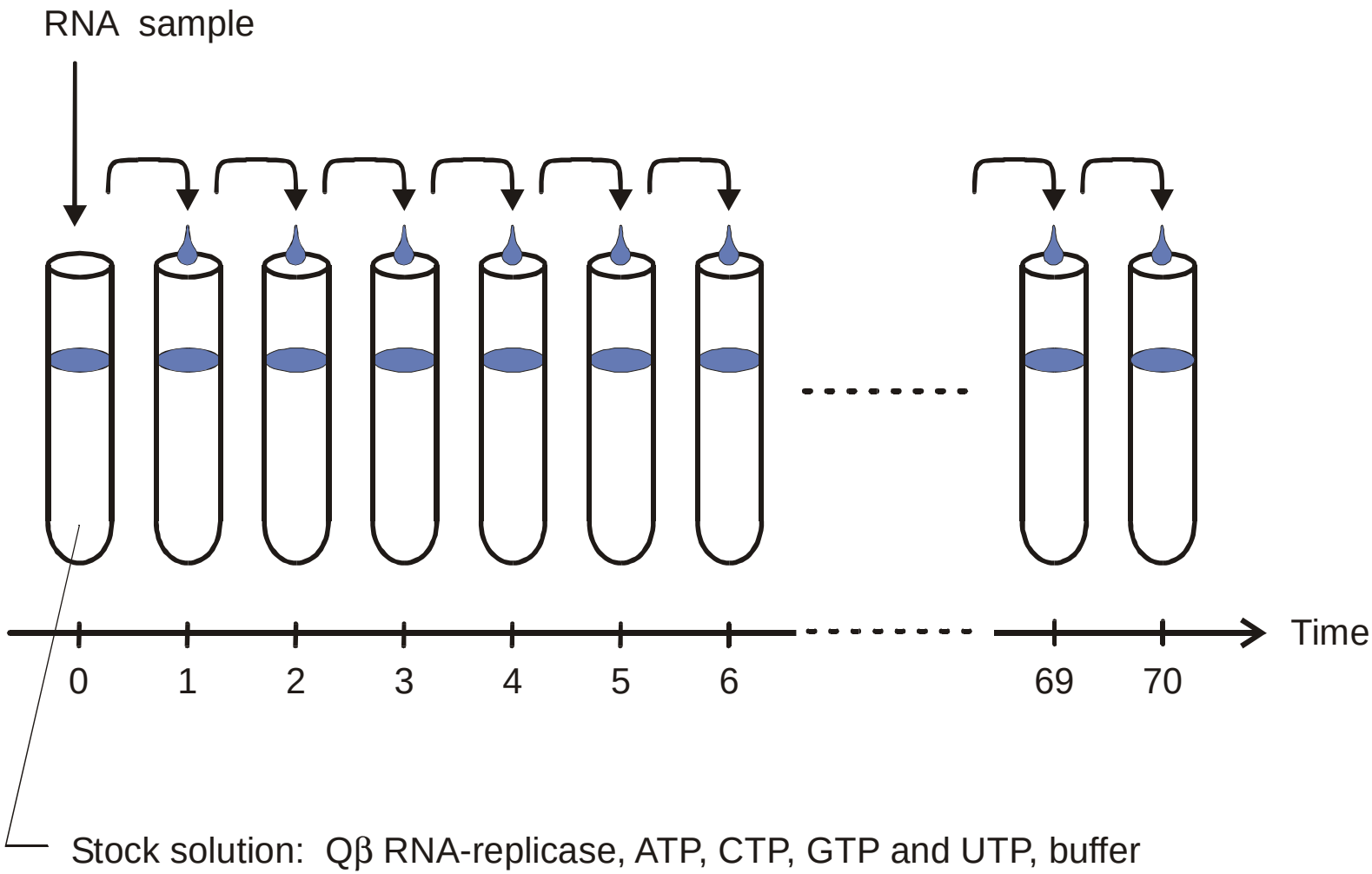

Anwendung der seriellen Überimpfungstechnik auf RNA-Evolution in Reagenzglas

Evolutionary design of RNA molecules

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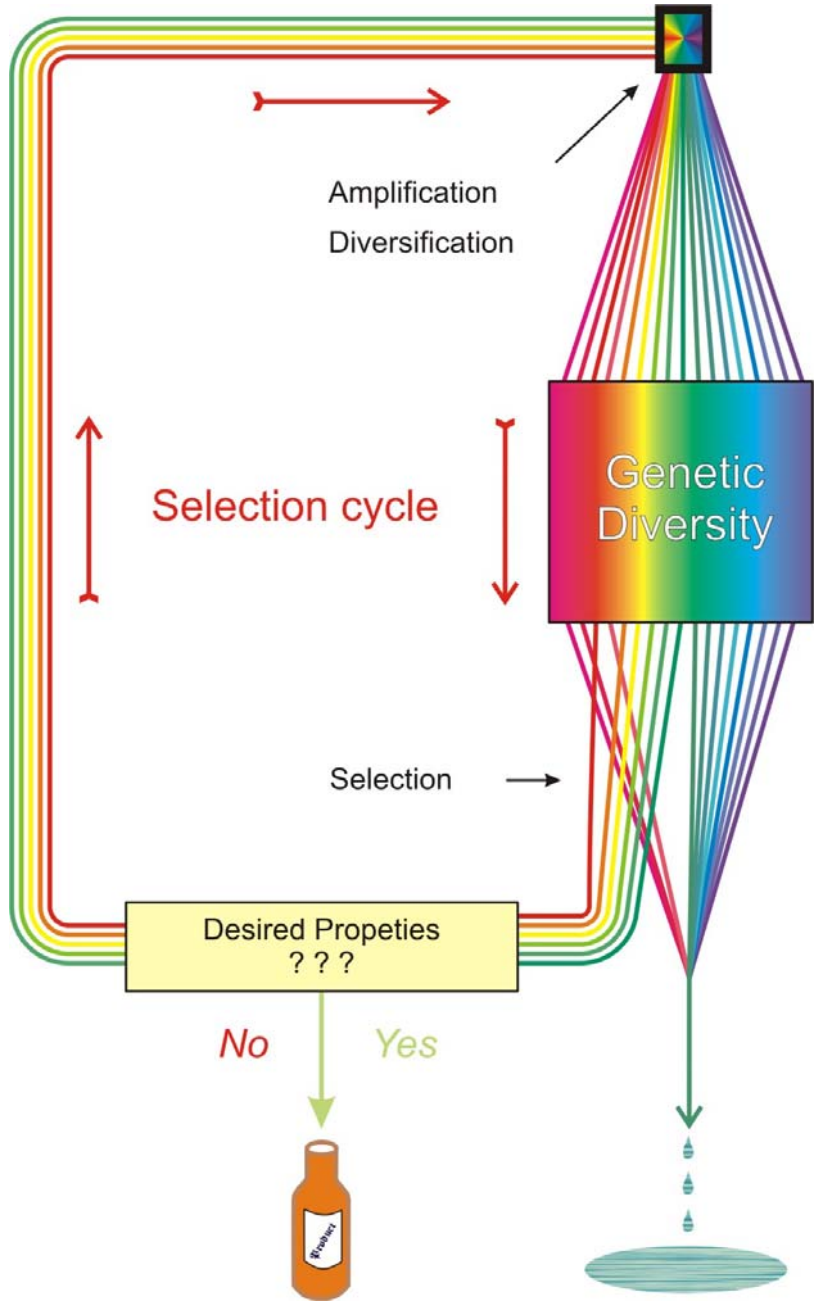
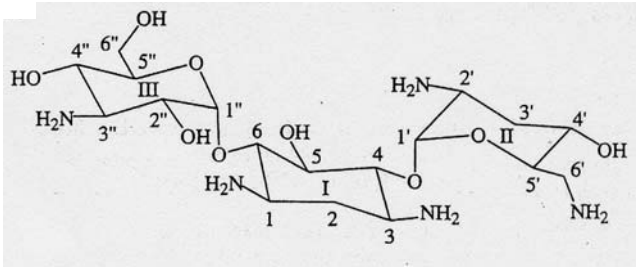
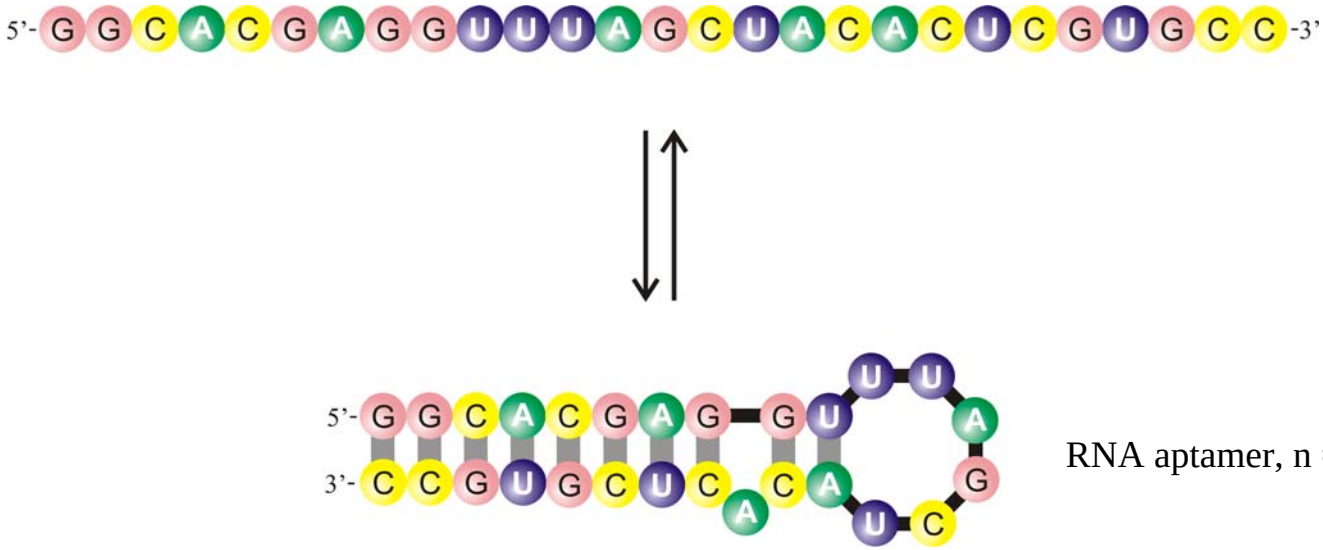
An example of ‘artificial selection’ with RNA molecules or ‘breeding’ of biomolecules





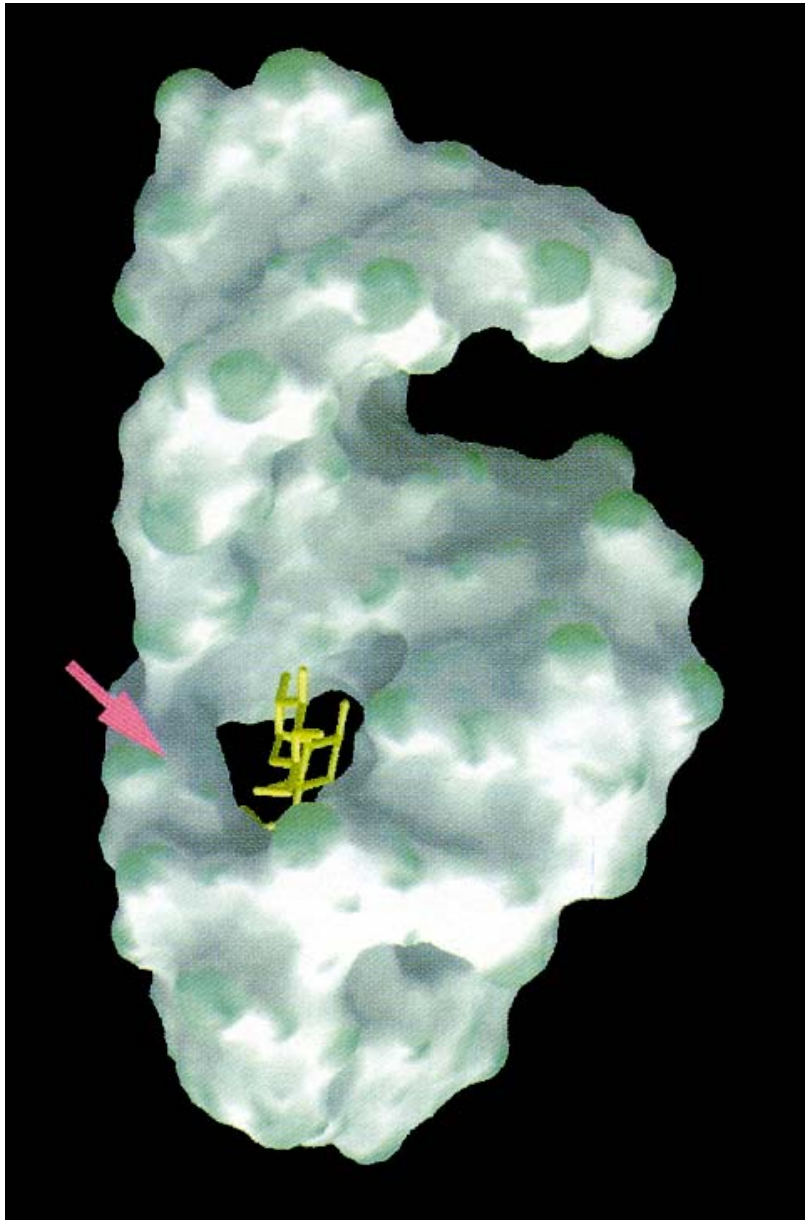
tobramycin



RNA aptamer, n = 27

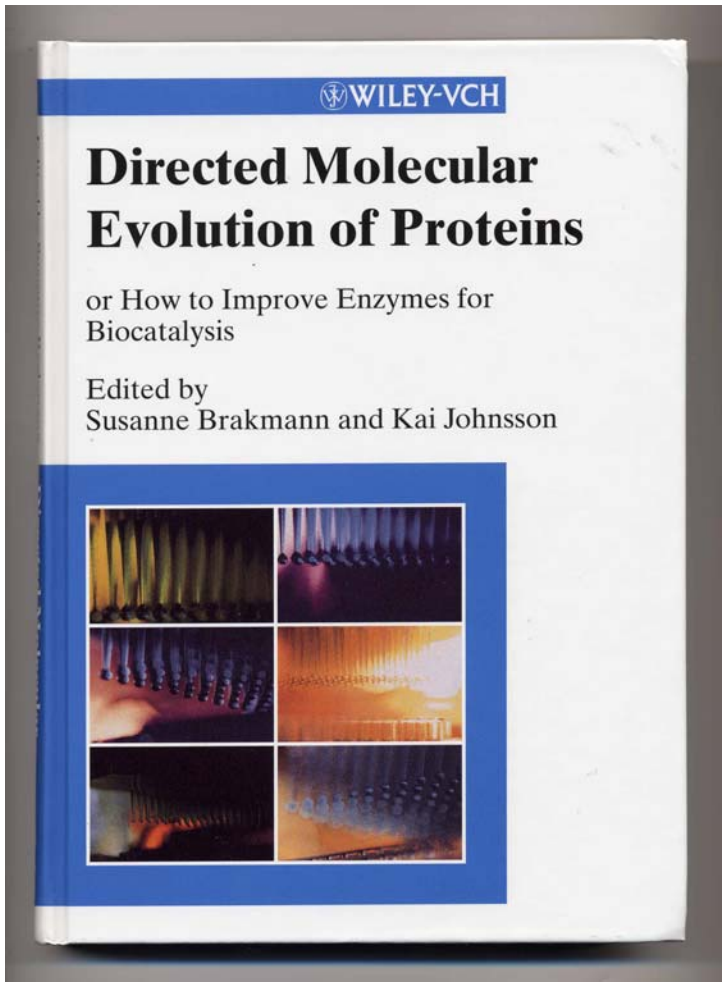
Formation of secondary structure of the tobramycin binding RNA aptamer with **K_D = 9 nM**

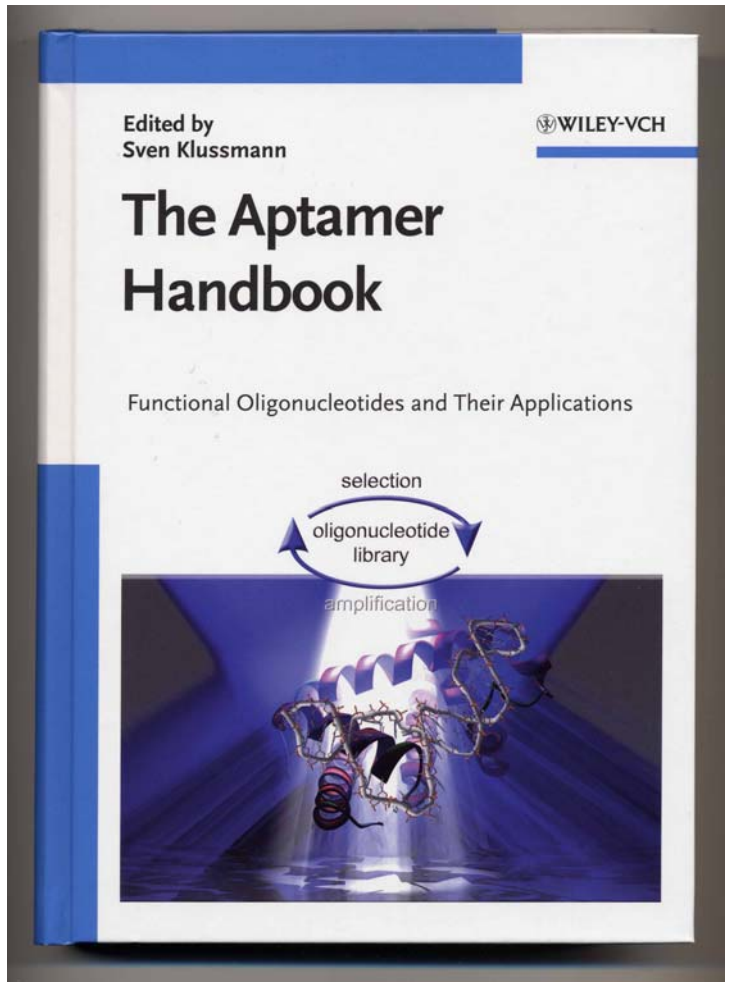
L. Jiang, A. K. Suri, R. Fiala, D. J. Patel, ***Saccharide-RNA recognition in an aminoglycoside antibiotic-RNA aptamer complex.*** Chemistry & Biology **4**:35-50 (1997)



The three-dimensional structure of the tobramycin aptamer complex

L. Jiang, A. K. Suri, R. Fiala, D. J. Patel, Chemistry & Biology **4**:35-50 (1997)





Application of molecular evolution to problems in biotechnology

Artificial evolution in biotechnology and pharmacology

G.F. Joyce. 2004. Directed evolution of nucleic acid enzymes. *Annu.Rev.Biochem*. **73**:791-836.

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S.J. Wrenn and P.B. Harbury. 2007. Chemical evolution as a tool for molecular discovery. *Annu.Rev.Biochem*. **76**:331-349.

1. Charles Darwin heute
2. Darwins Prinzip der natürlichen Auslese
3. Vermehrung von Molekülen
4. Chemische Kinetik der molekularen Evolution
5. Evolutionsexperimente mit Molekülen
6. **Simulation der Optimierung von Strukturen**
7. Ursachen und Konsequenzen der Neutralität

Evolution *in silico*

W. Fontana, P. Schuster,
Science **280** (1998), 1451-1455

random individuals. The primer pair used for genomic DNA amplification is 5′-TCTCCCTGGATTCTCATTTA-3′ (forward) and 5′-TCTTTGTCTTCTGTTCCACC-3′ (reverse). Reactions were performed in 25 μl using 1 unit of Taq DNA polymerase with each primer at 0.4 μM; 200 μM each dATP, dTTP, dGTP, and dCTP; and PCR buffer [10 mM tris-HCl (pH 8.3), 50 mM KCl_2,1.5 mM $MgCl_2$] in a cycle condition of 94°C for 1 min and then 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s followed by 72°C for 6 min. PCR products were purified (Qiagen), digested with Xmn I, and separated in a 2% agarose gel.

32. A nonsense mutation may affect mRNA stability and result in degradation of the transcript [L. Maquat, *Am. J. Hum. Genet.* **59**, 279 (1996)].
33. Data not shown; a dot blot with poly (A)$^+$ RNA from 50 human tissues (The Human RNA Master Blot, 7770-1, Clontech Laboratories) was hybridized with a probe from exons 29 to 47 of *MYO15* using the same condition as Northern blot analysis (*13*).
34. Smith–Magenis syndrome (SMS) is due to deletions of 17p11.2 of various sizes, the smallest of which includes *MYO15* and perhaps 20 other genes [(*6*); K-S Chen, L. Potocki, J. R. Lupski, *MRDD Res. Rev.* **2**, 122 (1996)]. *MYO15* expression is easily detected in the pituitary gland (data not shown). Haploinsufficiency for *MYO15* may explain a portion of the SMS phenotype such as short stature. Moreover, a few SMS patients have sensorineural hearing loss, possibly because of a point mutation in *MYO15* in trans to the SMS 17p11.2 deletion.
35. R. A. Fridell, data not shown.
36. K. B. Avraham *et al.*, *Nature Genet.* **11**, 369 (1995); X-Z. Liu *et al.*, *ibid.* **17**, 268 (1997); F. Gibson *et al.*, *Nature* **374**, 62 (1995); D. Weil *et al.*, *ibid.*, p. 60.
37. RNA was extracted from cochlea (membranous labyrinths) obtained from human fetuses at 18 to 22 weeks of development in accordance with guidelines established by the Human Research Committee at the Brigham and Women's Hospital. Only samples without evidence of degradation were pooled for poly (A)$^+$ selection over oligo(dT) columns. First-strand cDNA was prepared using an Advantage RT-for-PCR kit (Clontech Laboratories). A portion of the first-strand cDNA (4%) was amplified by PCR with Advantage cDNA polymerase mix (Clontech Laboratories) using human *MYO15*-specific oligonucleotide primers (forward, 5′-GCATGACCTGCCGGCTAATGGG-3′; reverse, 5′-CTCACGGCTTCTGCATGGTGCTCGGCTGGC-3′). Cycling conditions were 40 s at 94°C; 40 s at 66°C (3 cycles), 60°C (5 cycles), and 55°C (29 cycles); and 45 s at 68°C. PCR products were visualized by ethidium bromide staining after fractionation in a 1% agarose gel. A 688-bp PCR product is expected from amplification of the human *MYO15* cDNA. Amplification of human genomic DNA with this primer pair would result in a 2903-bp fragment.
38. We are grateful to the people of Bengkala, Bali, and the two families from India. We thank J. R. Lupski and K.-S. Chen for providing the human chromosome 17 cosmid library. For technical and computational assistance, we thank N. Dietrich, M. Fergusson, A. Gupta, E. Sorbello, R. Torkzadeh, C. Varner, M. Walker, G. Bouffard, and S. Beckstrom-Sternberg (National Institutes of Health Intramural Sequencing Center). We thank J. T. Hinnant, I. N. Arhya, and S. Winata for assistance in Bali, and T. Barber, S. Sullivan, E. Green, D. Drayna, and J. Battey for helpful comments on this manuscript. Supported by the National Institute on Deafness and Other Communication Disorders (NIDCD) (Z01 DC 00035-01 and Z01 DC 00038-01 to T.B.F. and E.R.W. and R01 DC 03402 to C.C.M.), the National Institute of Child Health and Human Development (R01 HD30428 to S.A.C.) and a National Science Foundation Graduate Research Fellowship to F.J.P. This paper is dedicated to J. B. Snow Jr. on his retirement as the Director of the NIDCD.

9 March 1998; accepted 17 April 1998

Continuity in Evolution: On the Nature of Transitions

Walter Fontana and Peter Schuster

To distinguish continuous from discontinuous evolutionary change, a relation of nearness between phenotypes is needed. Such a relation is based on the probability of one phenotype being accessible from another through changes in the genotype. This nearness relation is exemplified by calculating the shape neighborhood of a transfer RNA secondary structure and provides a characterization of discontinuous shape transformations in RNA. The simulation of replicating and mutating RNA populations under selection shows that sudden adaptive progress coincides mostly, but not always, with discontinuous shape transformations. The nature of these transformations illuminates the key role of neutral genetic drift in their realization.

A much-debated issue in evolutionary biology concerns the extent to which the history of life has proceeded gradually or has been punctuated by discontinuous transitions at the level of phenotypes (*1*). Our goal is to make the notion of a discontinuous transition more precise and to understand how it arises in a model of evolutionary adaptation.

We focus on the narrow domain of RNA secondary structure, which is currently the simplest computationally tractable, yet realistic phenotype (*2*). This choice enables the definition and exploration of concepts that may prove useful in a wider context. RNA secondary structures represent a coarse level of analysis compared with the three-dimensional structure at atomic resolution. Yet, secondary structures are empirically well defined and obtain their biophysical and biochemical importance from being a scaffold for the tertiary structure. For the sake of brevity, we shall refer to secondary structures as "shapes." RNA combines in a single molecule both genotype (replicatable sequence) and phenotype (selectable shape), making it ideally suited for in vitro evolution experiments (*3*, *4*).

To generate evolutionary histories, we used a stochastic continuous time model of an RNA population replicating and mutating in a capacity-constrained flow reactor under selection (*5*, *6*). In the laboratory, a goal might be to find an RNA aptamer binding specifically to a molecule (*4*). Although in the experiment the evolutionary end product was unknown, we thought of its shape as being specified implicitly by the imposed selection criterion. Because our intent is to study evolutionary histories rather than end products, we defined a target shape in advance and assumed the replication rate of a sequence to be a function of the similarity between its shape and the target. An actual situation may involve more than one best shape, but this does not affect our conclusions.

An instance representing in its qualitative features all the simulations we performed is shown in Fig. 1A. Starting with identical sequences folding into a random shape, the simulation was stopped when the population became dominated by the target, here a canonical tRNA shape. The black curve traces the average distance to the target (inversely related to fitness) in the population against time. Aside from a short initial phase, the entire history is dominated by steps, that is, flat periods of no apparent adaptive progress, interrupted by sudden approaches toward the target structure (*7*). However, the dominant shapes in the population not only change at these marked events but undergo several fitness-neutral transformations during the periods of no apparent progress. Although discontinuities in the fitness trace are evident, it is entirely unclear when and on the basis of what the series of successive phenotypes itself can be called continuous or discontinuous.

A set of entities is organized into a (topological) space by assigning to each entity a system of neighborhoods. In the present case, there are two kinds of entities: sequences and shapes, which are related by a thermodynamic folding procedure. The set of possible sequences (of fixed length) is naturally organized into a space because point mutations induce a canonical neighborhood. The neighborhood of a sequence consists of all its one-error mutants. The problem is how to organize the set of possible shapes into a space. The issue arises because, in contrast to sequences, there are

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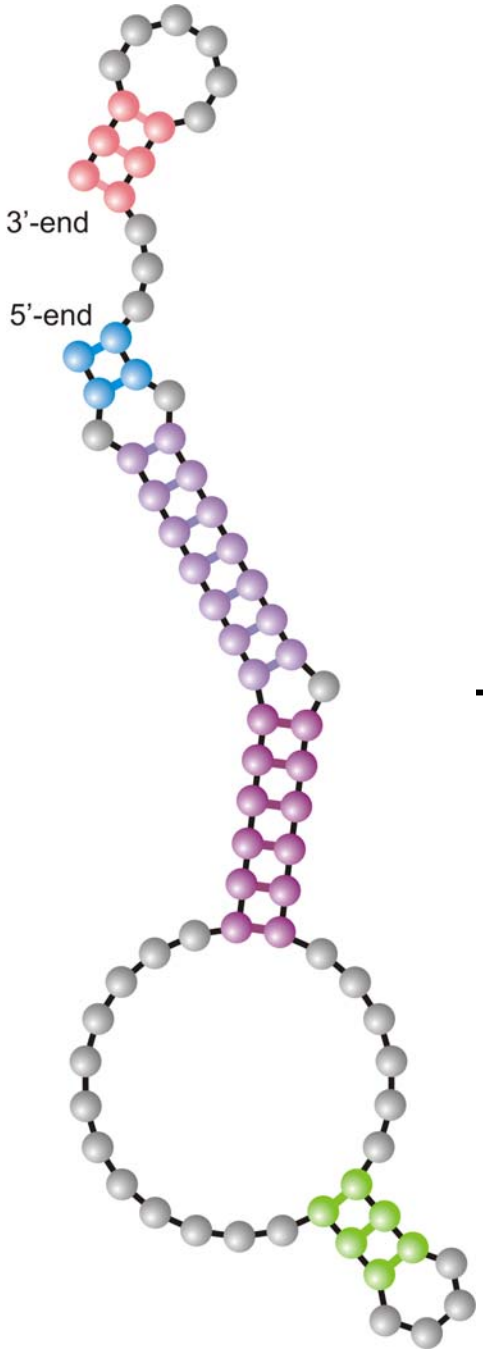
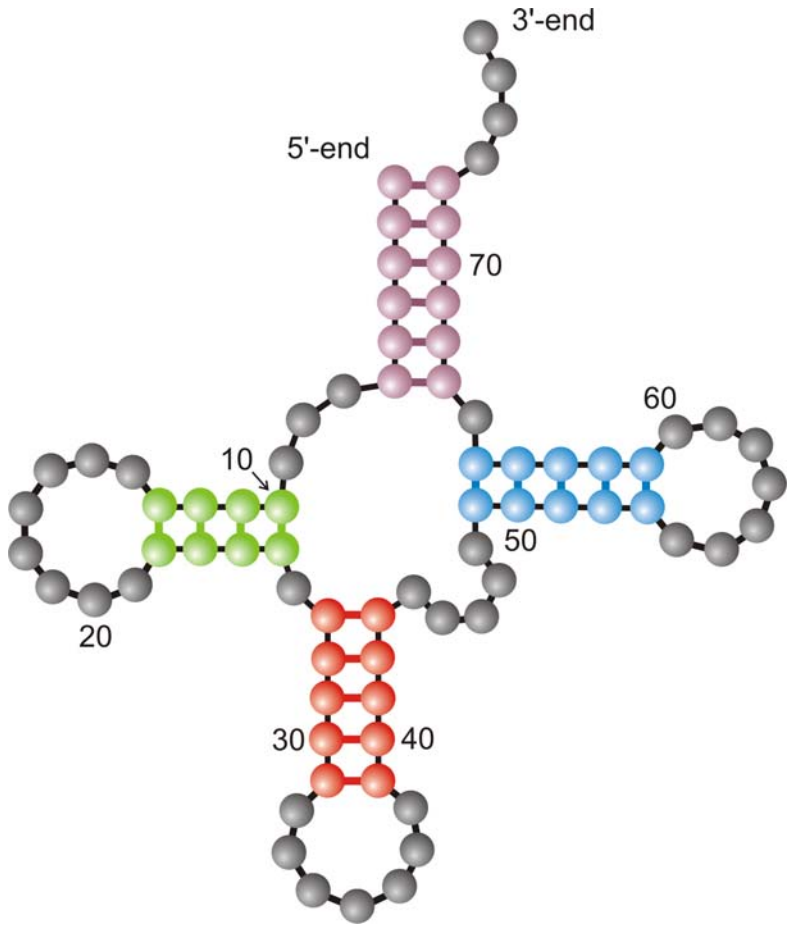
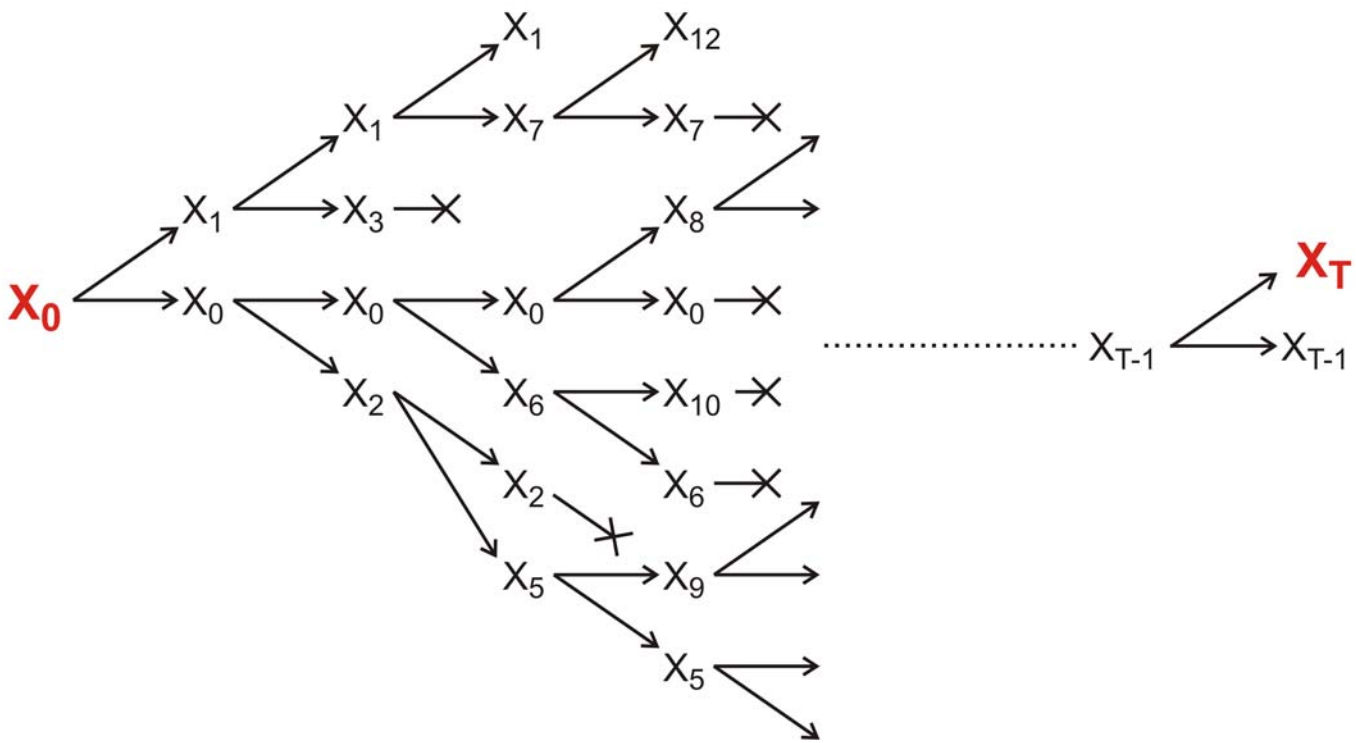


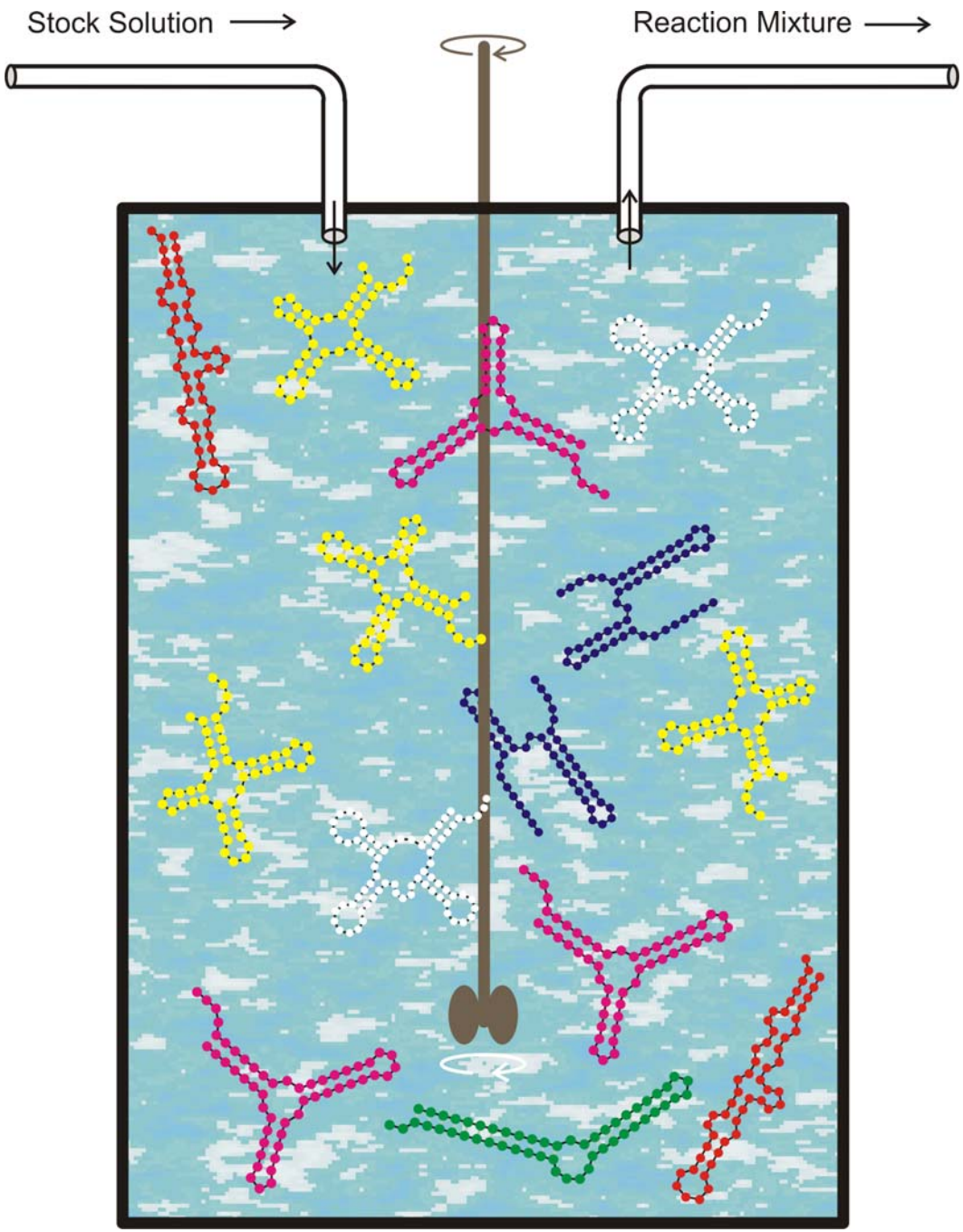
Structure of randomly chosen initial sequence



Phenylalanyl-tRNA as target structure



Evolution of RNA molecules as a Markow process and its analysis by means of the relay series



Replication rate constant

(Fitness):

$$f_k = \gamma / [\alpha + \Delta d_S^{(k)}]$$

$$\Delta d_S^{(k)} = d_H(S_k, S_\tau)$$

Selection pressure:

The population size,

N = # RNA moleucles,

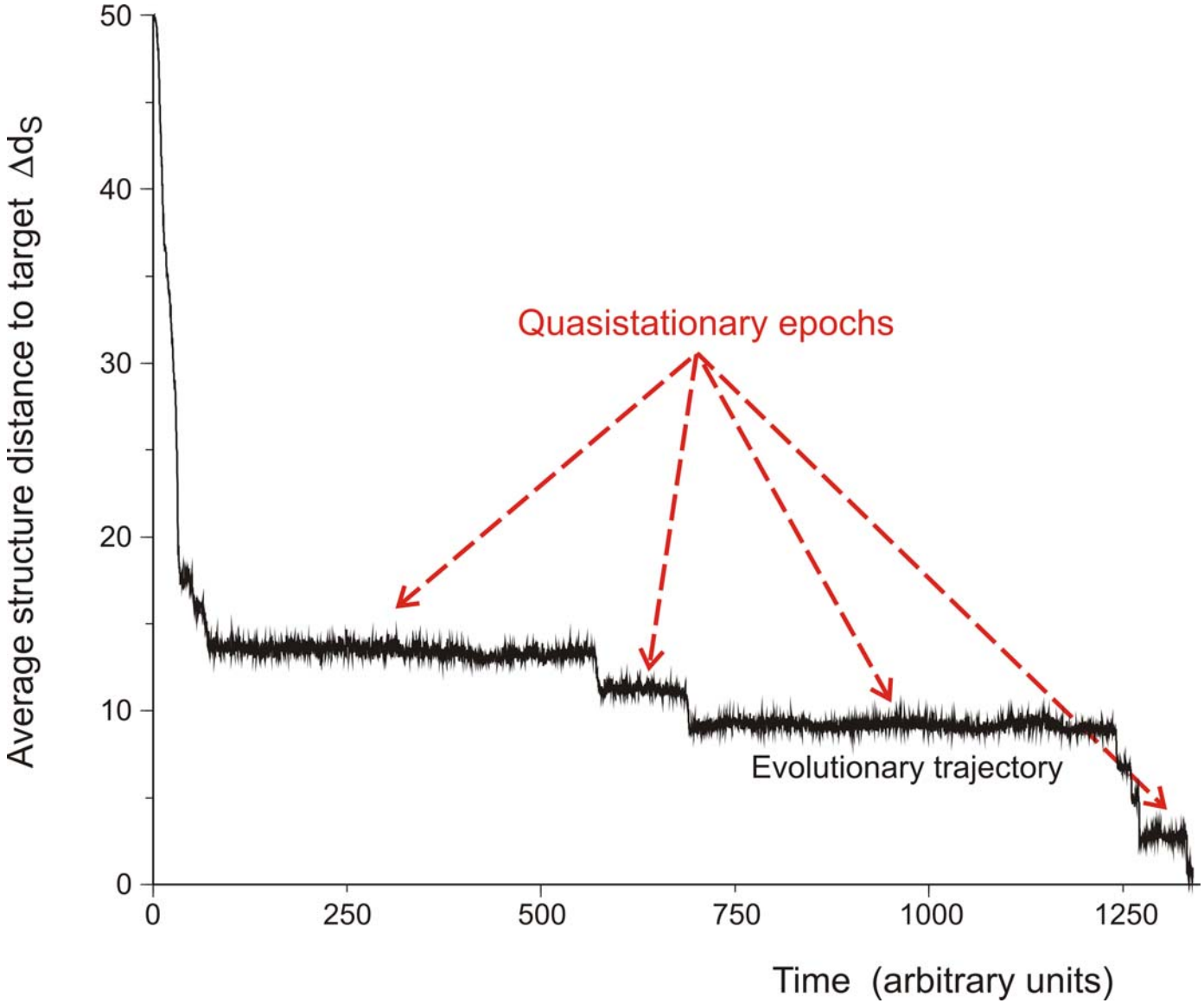
is determined by the flux:

$$N(t) \approx \overline{N} \pm \sqrt{\overline{N}}$$

Mutation rate:

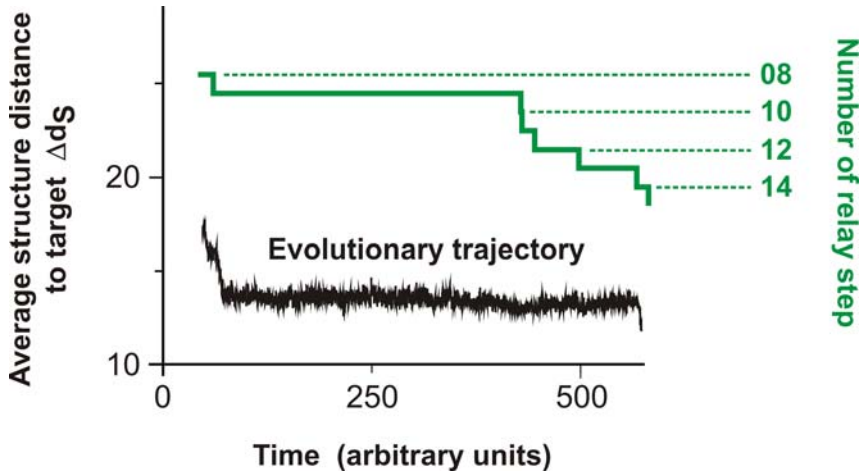
p = 0.001 / Nucleotide × Replication

The flow reactor as a device for studying the evolution of molecules ***in vitro*** and ***in silico***.



In silico optimization in the flow reactor: Evolutionary Trajectory

28 neutral point mutations during a long quasi-stationary epoch



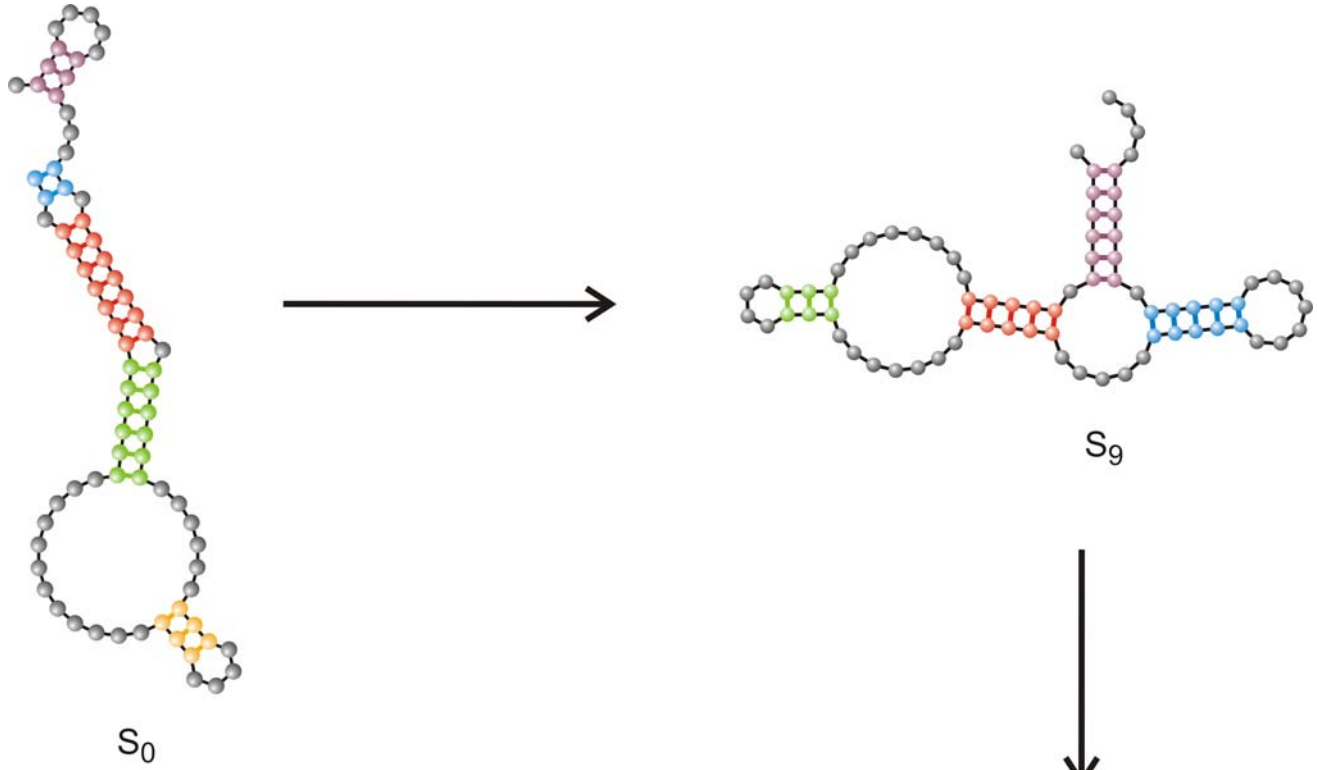

```
entry   GGUAUGGGCGUUGAAUAGUAGGGUUUAAACCAAUCGGCCAACGAUCUCGUGUGCGCAUUUCAUAUCCCGUACAGAA
8       .((((((((((((.........(((....)))).......)))))).....((((.......))))))))))....
exit    GGUAUGGGCGUUGAAUAAUAGGGUUUAAACCAAUCGGCCAACGAUCUCGUGUGCGCAUUUCAUAUCCCAUACAGAA
entry   GGUAUGGGCGUUGAAUAAUAGGGUUUAAACCAAUCGGCCAACGAUCUCGUGUGCGCAUUUCAUAUACCAUACAGAA
9       .((((((.(((((.........(((....)))).......)))))).....((((.......))))).))))))....
exit    UGGAUGGACGUUGAAUAACAAGGUAUCGACCAAACAACCAACGAGUAAGUGUGUACGCCCCACACACCGUCCCAAG
entry   UGGAUGGACGUUGAAUAACAAGGUAUCGACCAAACAACCAACGAGUAAGUGUGUACGCCCCACACAGCGUCCCAAG
10      .(((((..(((((.........(((....)))).......)))))).....((((.......))))).))))))....
exit    UGGAUGGACGUUGAAUAACAAGGUAUCGACCAAACAACCAACGAGUAAGUGUGUACGCCCCACACAGCGUCCCAAG
```

Transition inducing point mutations change the molecular structure

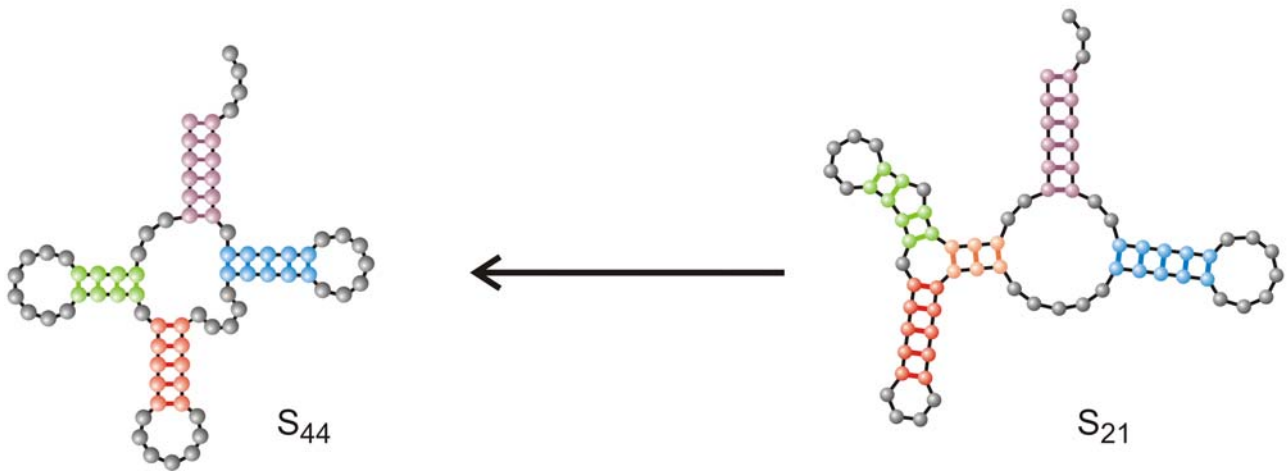
Neutral point mutations leave the molecular structure unchanged

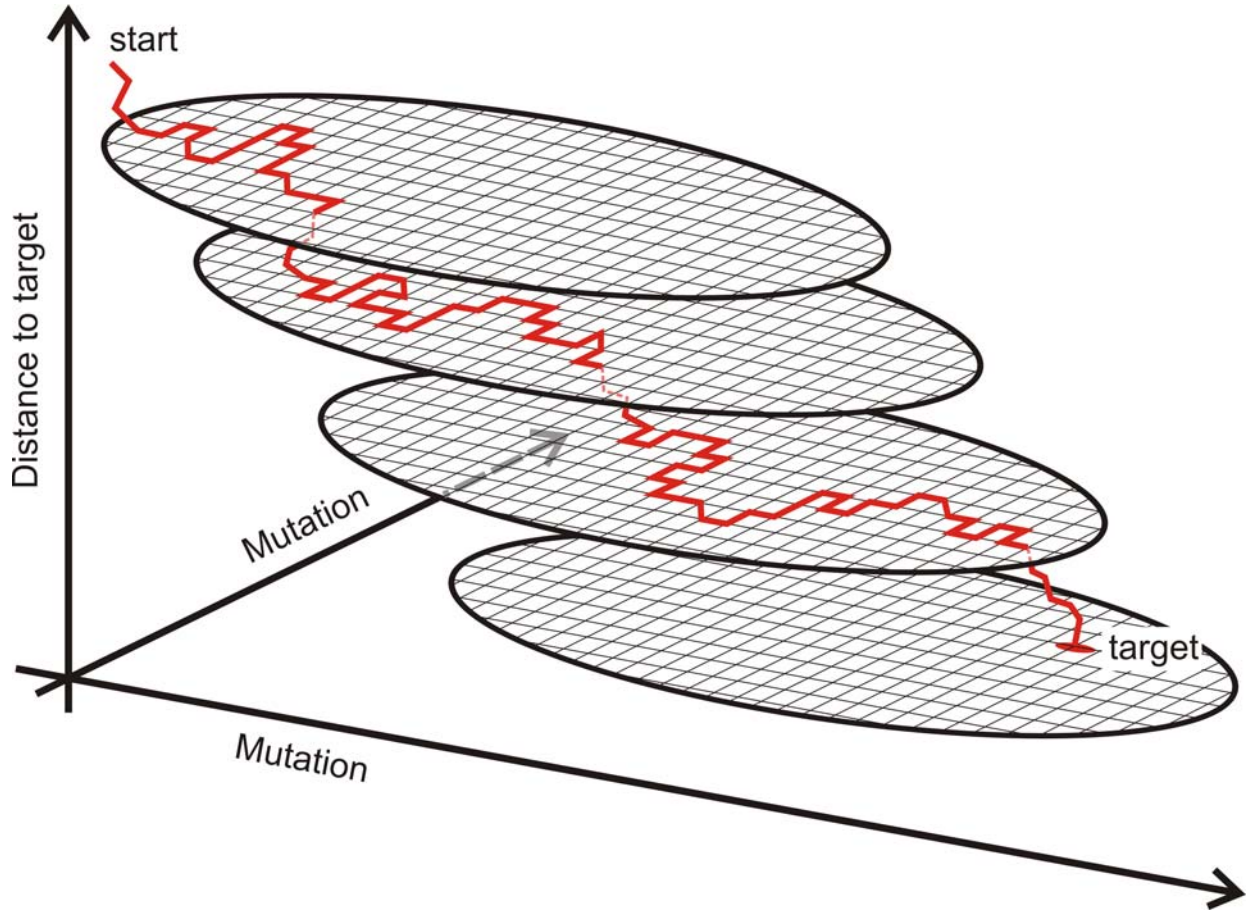
Neutral genotype evolution during phenotypic stasis

Randomly chosen
initial structure



Phenylalanyl-tRNA
as target structure





A sketch of optimization on neutral networks

1. Charles Darwin heute
2. Darwins Prinzip der natürlichen Auslese
3. Vermehrung von Molekülen
4. Chemische Kinetik der molekularen Evolution
5. Evolutionsexperimente mit Molekülen
6. Simulation der Optimierung von Strukturen
7. **Ursachen und Konsequenzen der Neutralität**

Was bedeutet Neutralität ?

Selektive Neutralität =
= mehrere Genotypen weisen identische Fitness auf.

Strukturelle Neutralität =
= mehrere Genotypen bilden identische Strukturen aus.

ON

THE ORIGIN OF SPECIES

BY MEANS OF NATURAL SELECTION,

OR THE

PRESERVATION OF FAVOURED RACES IN THE STRUGGLE FOR LIFE.

By CHARLES DARWIN, M.A.,

FELLOW OF THE ROYAL, GEOLOGICAL, LINNÆAN, ETC., SOCIETIES;
AUTHOR OF 'JOURNAL OF RESEARCHES DURING H. M. S. BEAGLE'S VOYAGE ROUND THE WORLD.'

LONDON:
JOHN MURRAY, ALBEMARLE STREET.
1859.

This preservation of favourable individual differences and variations, and the destruction of those which are injurious, I have called Natural Selection, or the Survival of the Fittest. Variations neither useful nor injurious would not be affected by natural selection, and would be left either a fluctuating element, as perhaps we see in certain polymorphic species, or would ultimately become fixed, owing to the nature of the organism and the nature of the conditions.

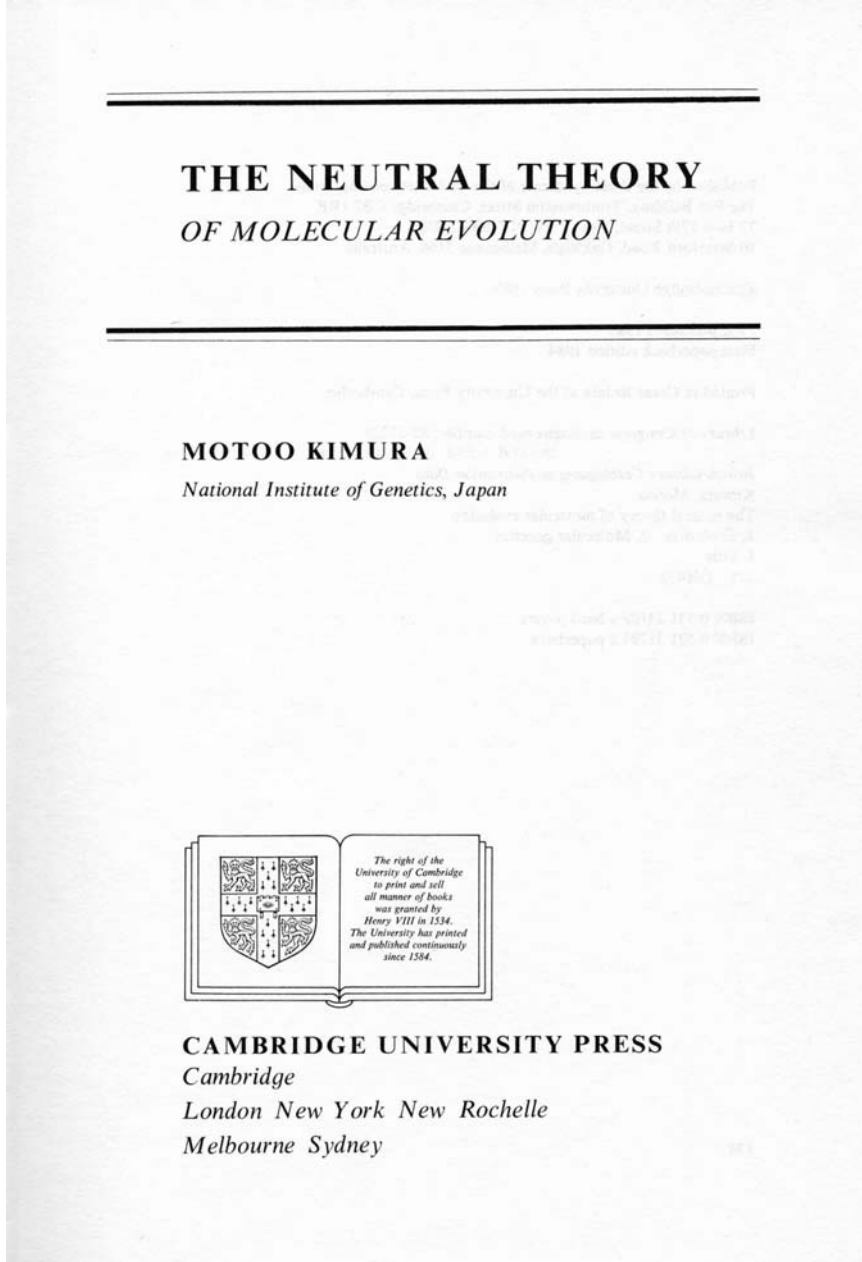
Charles Darwin. *The Origin of Species*. Sixth edition. John Murray. London: 1872

MOTOO KIMURA

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

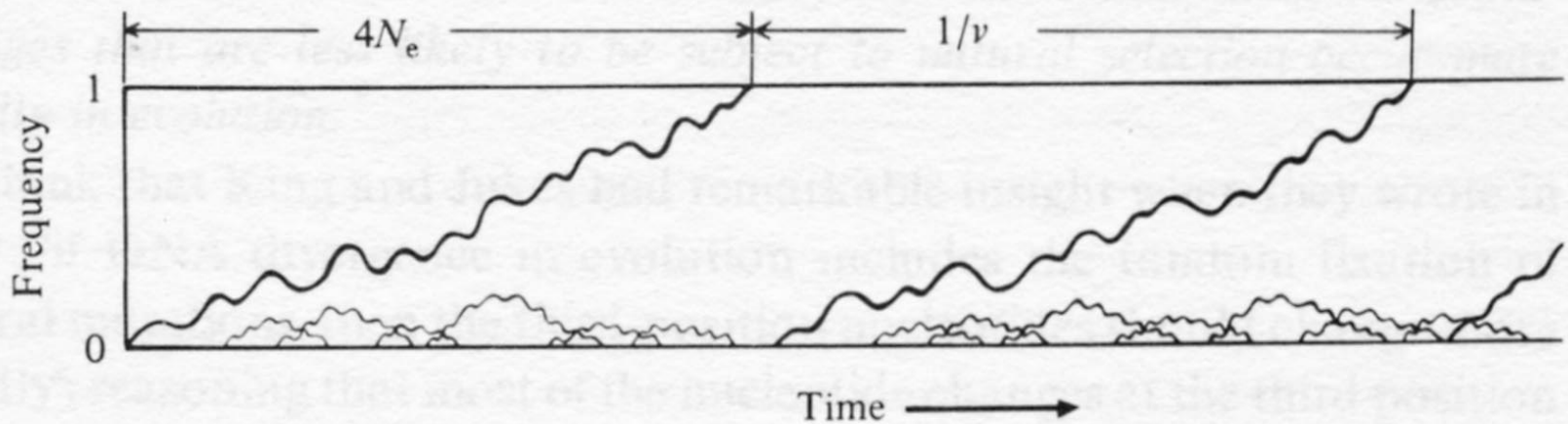
OF MOLECULAR EVOLUTION

MOTOO KIMURA

National Institute of Genetics, Japan

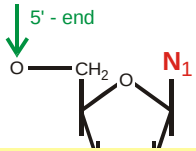
CAMBRIDGE UNIVERSITY PRESS
Cambridge
London New York New Rochelle
Melbourne Sydney

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.

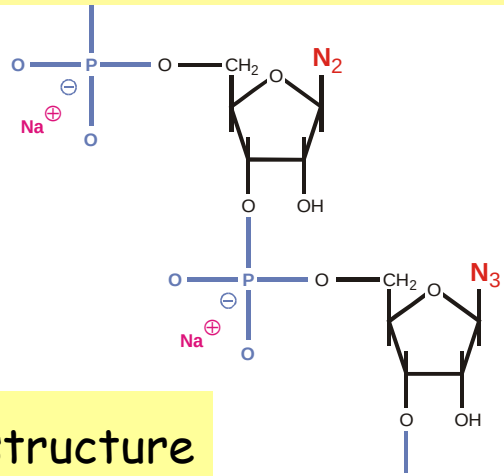


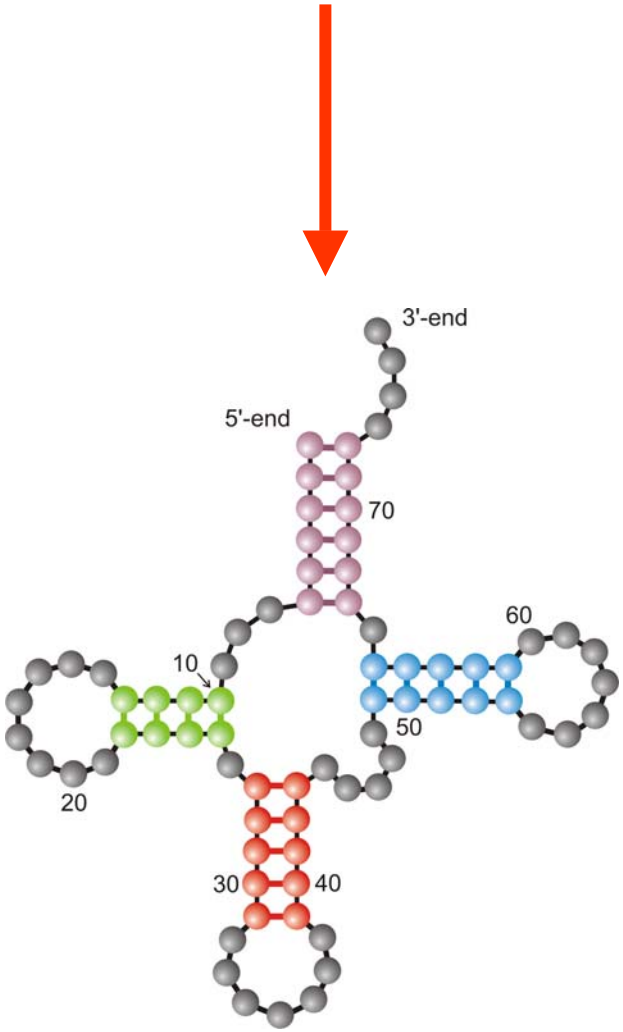
The average time of replacement of a dominant genotype in a population is the reciprocal mutation rate, 1/ν, and therefore independent of population size.

Fixation of mutants in neutral evolution (Motoo Kimura, 1955)

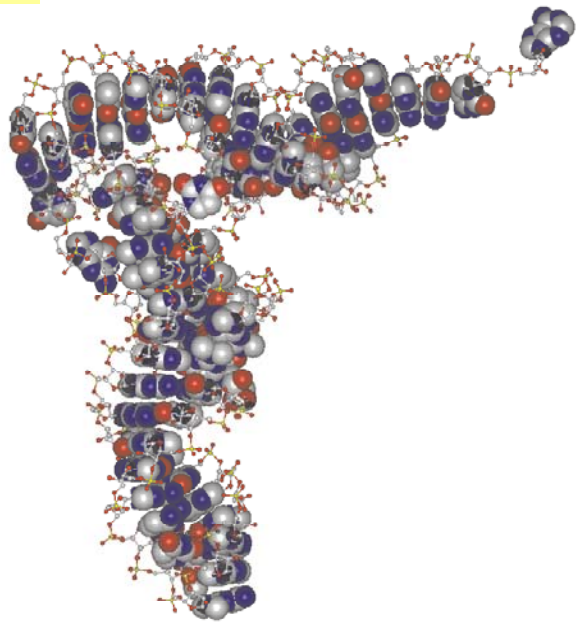


5'-end GCGGAUUUAGCUCAGUUGGGAGAGCGCCAGACUGAAGAUCUGGAGGUCCUGUGUUCGAUCCACAGAAUUCGCACCA 3'-end





Definition of RNA structure



RNA sequence: GUAUCGAAAUACGUAGCGUAUGGGGAUGCUGGACGGUCCCAUCGGUACUCCA

RNA folding:

Structural biology, spectroscopy of biomolecules, understanding **molecular function**

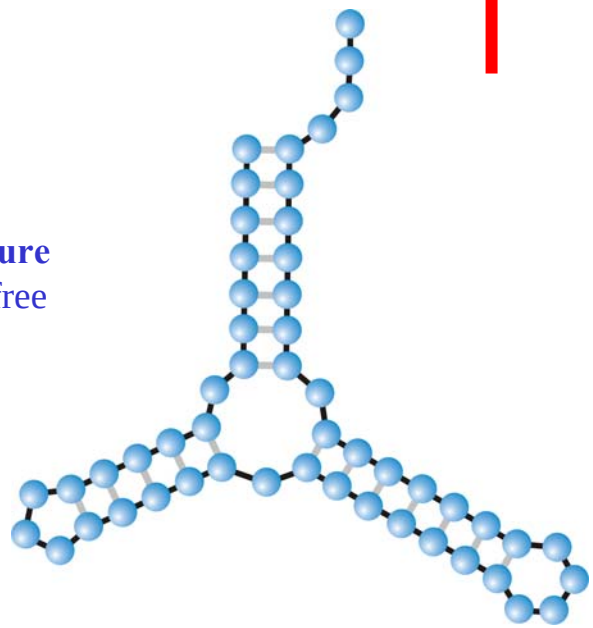
Iterative determination of a sequence for the given secondary structure

Inverse Folding Algorithm

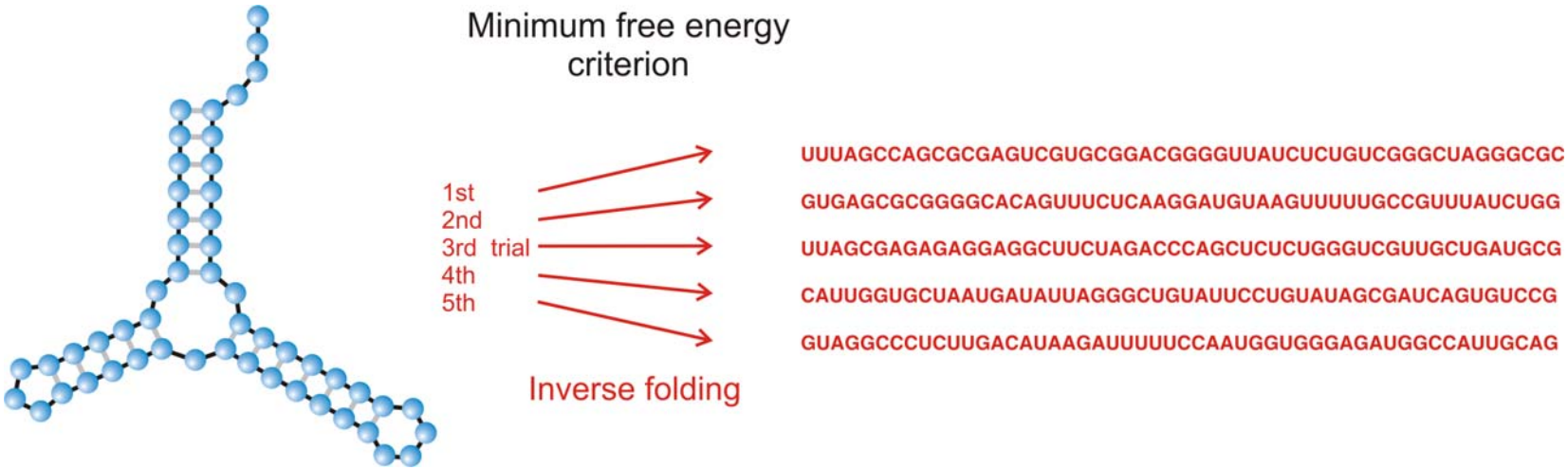
Inverse folding of RNA:

Biotechnology, **design of biomolecules** with predefined structures and functions

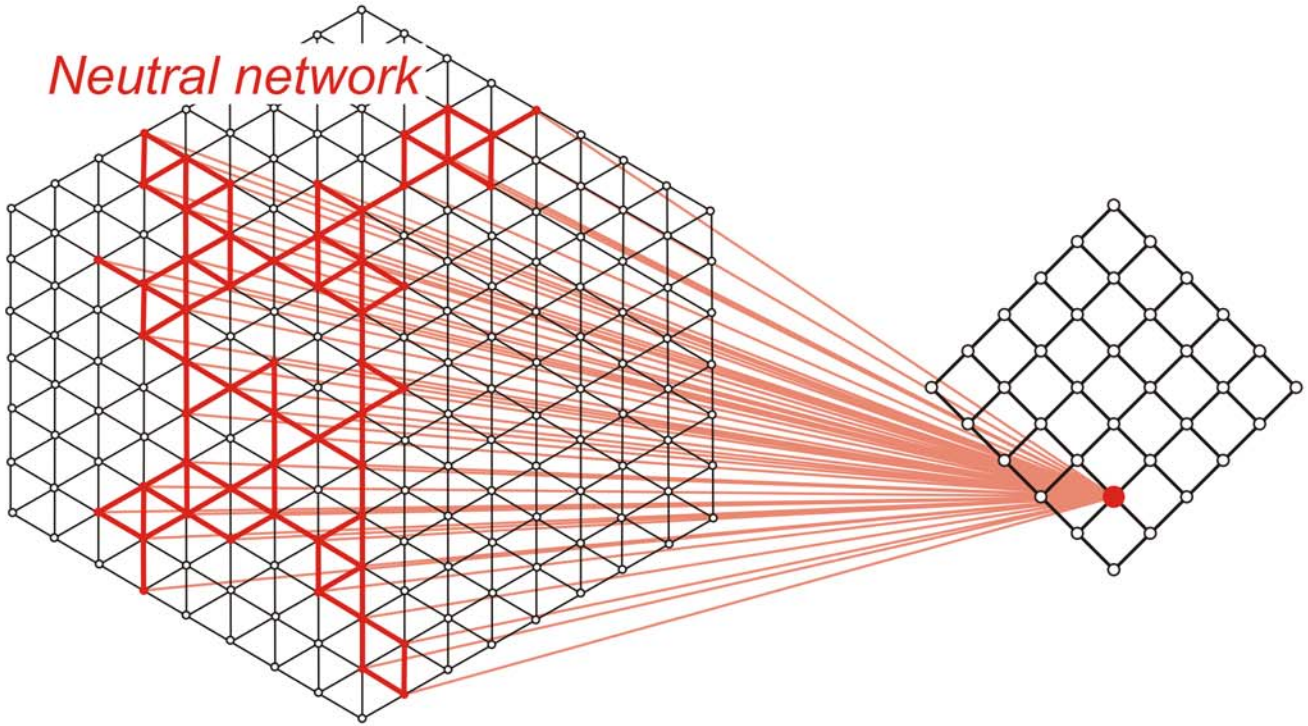
RNA structure of minimal free energy:



Sequence, structure, and design



The inverse folding algorithm searches for sequences that form a given RNA secondary structure under the minimum free energy criterion.



Sequence space

Structure space

many genotypes ⇒ one phenotype

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Rep. Prog. Phys. **69** (2006) 1419–1477 doi:10.1088/0034-4885/69/5/R04

Prediction of RNA secondary structures: from theory to models and real molecules

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STATIONARY MUTANT DISTRIBUTIONS AND EVOLUTIONARY OPTIMIZATION

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Molecular evolution is modelled by erroneous replication of binary sequences. We show how the selection of two species of equal or almost equal selective value is influenced by its nearest neighbours in sequence space. In the case of perfect neutrality and sufficiently small error rates we find that the Hamming distance between the species determines selection. As the error rate increases the fitness parameters of neighbouring species become more and more important. In the case of almost neutral sequences we observe a critical replication accuracy at which a drastic change in the "quasispecies", in the stationary mutant distribution occurs. Thus, in frequently mutating populations fitness turns out to be an ensemble property rather than an attribute of the individual.

In addition we investigate the time dependence of the mean excess production as a function of initial conditions. Although it is optimized under most conditions, cases can be found which are characterized by decrease or non-monotonous change in mean excess productions.

1. Introduction. Recent data from populations of RNA viruses provided direct evidence for vast sequence heterogeneity (Domingo *et al.*, 1987). The origin of this diversity is not yet completely known. It may be caused by the low replication accuracy of the polymerizing enzyme, commonly a virus specific, RNA dependent RNA synthetase, or it may be the result of a high degree of selective neutrality of polynucleotide sequences. Eventually, both factors contribute to the heterogeneity observed. Indeed, mutations occur much more frequently than previously assumed in microbiology. They are by no means rare events and hence, neither the methods of conventional population genetics (Ewens, 1979) nor the neutral theory (Kimura, 1983) can be applied to these virus populations. Selectively neutral variants may be close with respect to Hamming distance and then the commonly made assumption that the mutation backflow from the mutants to the wilde type is negligible does not apply.

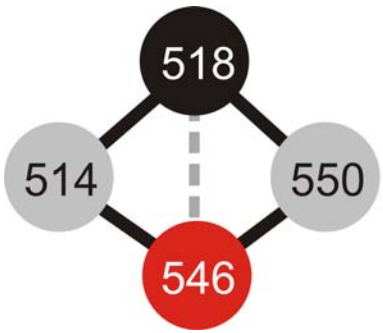
A kinetic theory of polynucleotide evolution which was developed during the past 15 years (Eigen, 1971; 1985; Eigen and Schuster, 1979; Eigen *et al.*, 1987; Schuster, 1986); Schuster and Sigmund, 1985) treats correct replication and mutation as parallel reactions within one and the same reaction network

Neutral network

$\lambda = 0.01,\ s = 367$

$\mathbf{d_H = 1}$

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



Neutral network

$\lambda = 0.01,\ s = 877$

$\mathbf{d_H = 2}$

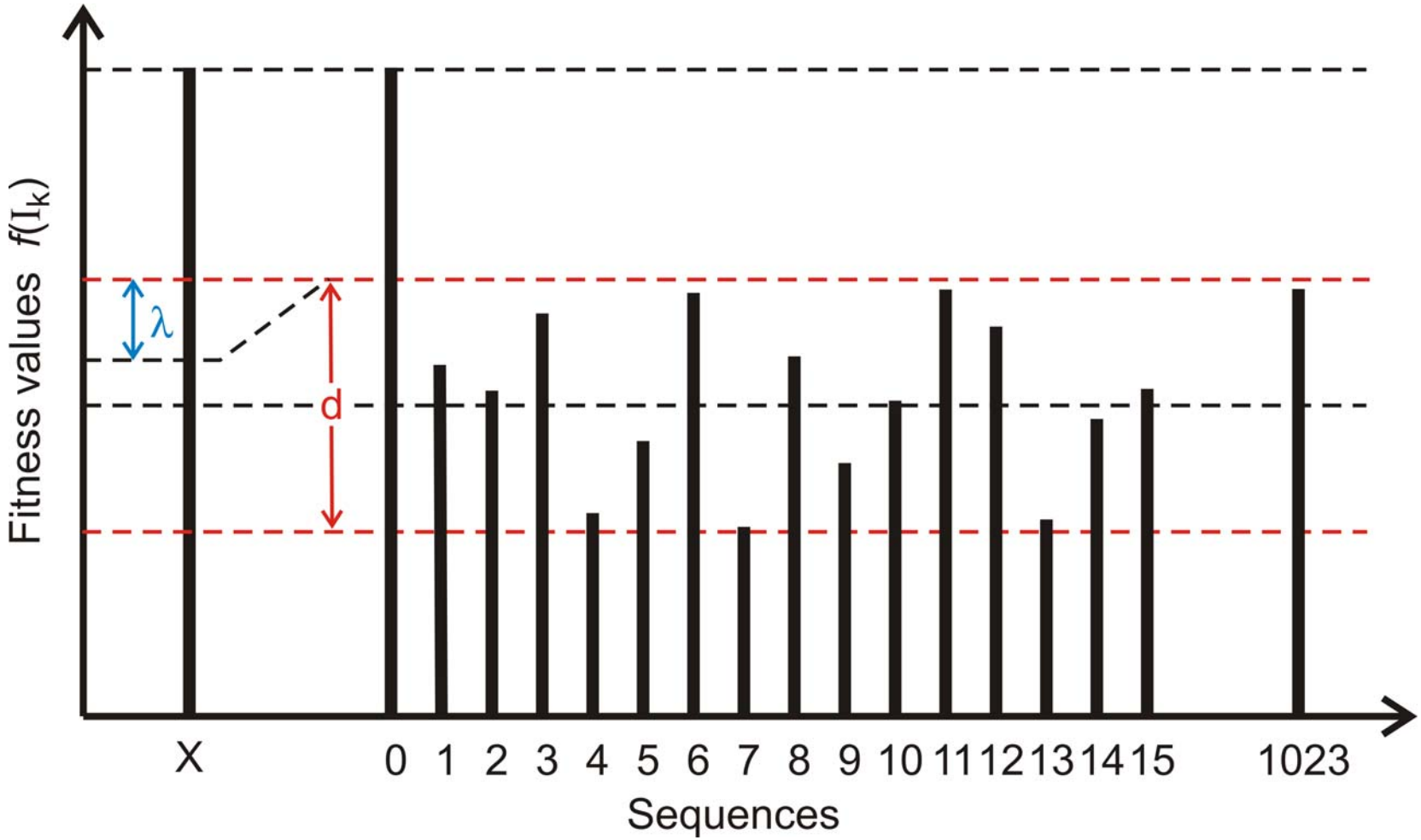
$$\lim_{p\to 0} x_1(p) = a$$

$$\lim_{p\to 0} x_2(p) = 1 - a$$

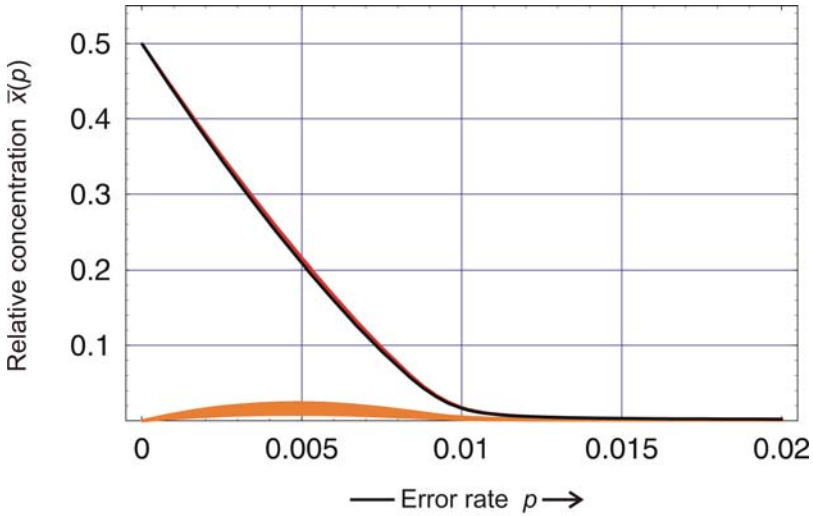
$\mathbf{d_H \geq 3}$

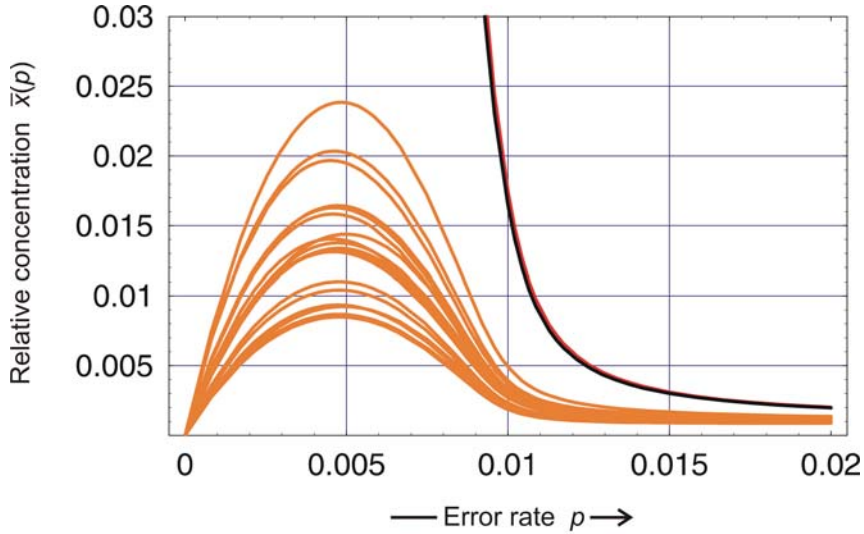
random fixation in the sense of Motoo Kimura

Pairs of genotypes in neutral replication networks



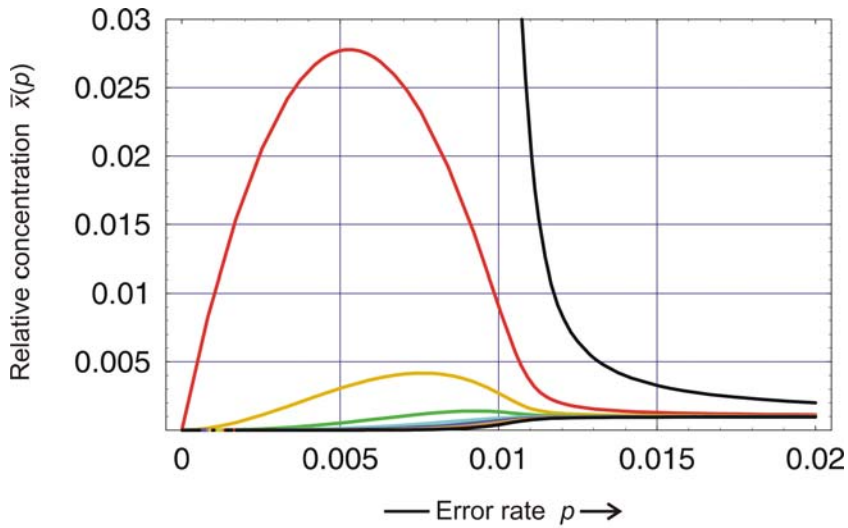
A fitness landscape including neutrality





Neutral network

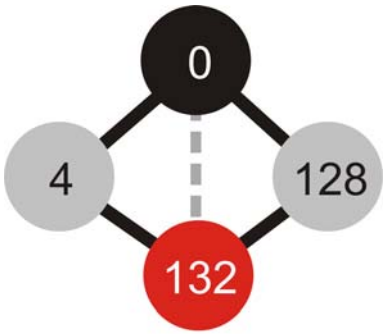
$\lambda = 0.01,\ s = 367$



Neutral network: Individual sequences

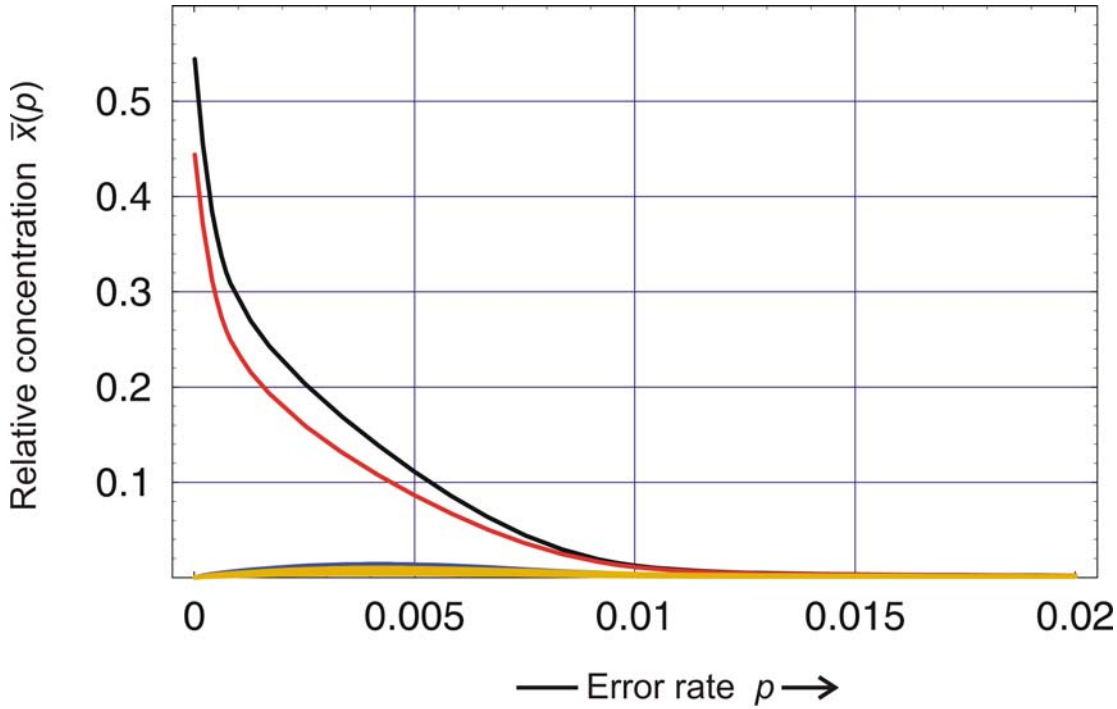
$n = 10,\ \sigma = 1.1,\ d = 1.0$

Consensus sequence of a quasispecies of two strongly coupled sequences of Hamming distance $d_H(X_{i,},X_j) = 1$.



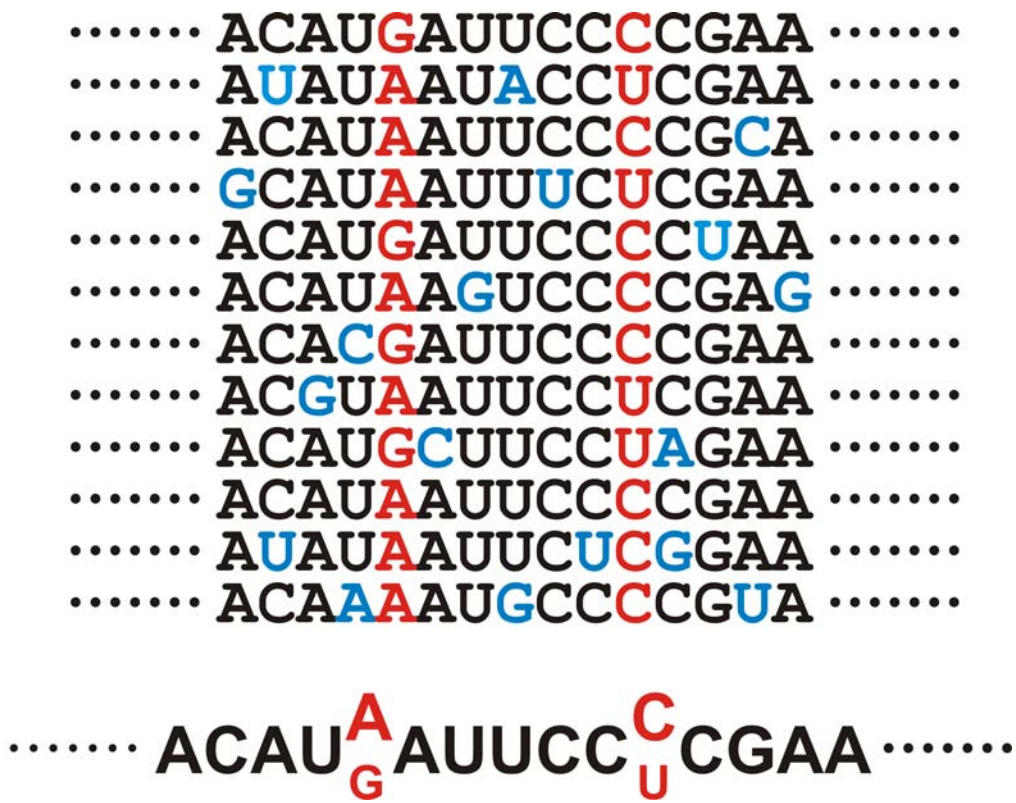
Neutral network

$\lambda = 0.01,\ s = 877$

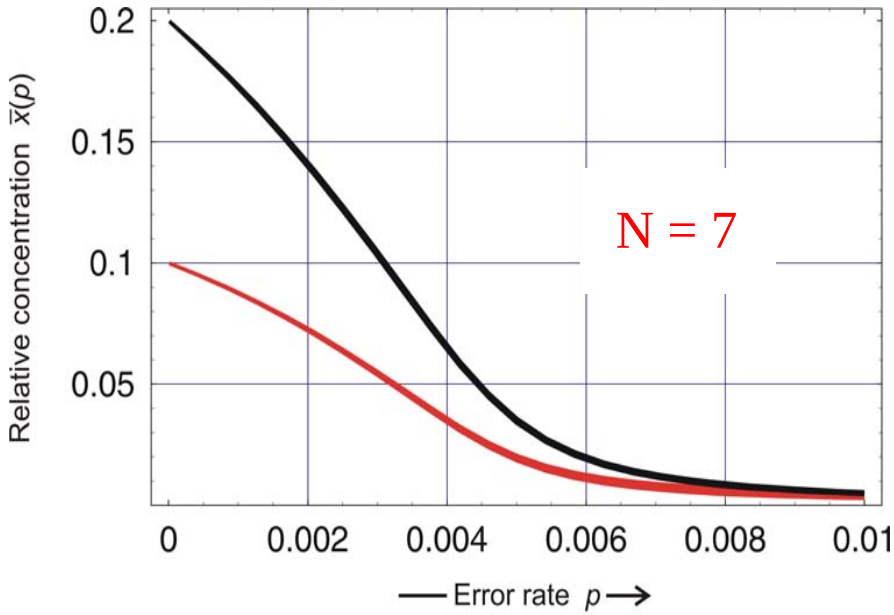


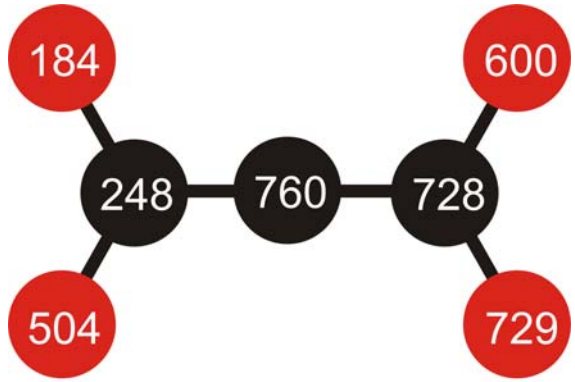
Neutral network: Individual sequences

n = 10, σ = 1.1, d = 1.0



Consensus sequence of a quasispecies of two strongly coupled sequences of Hamming distance $d_H(X_{i,},X_j) = 2$.

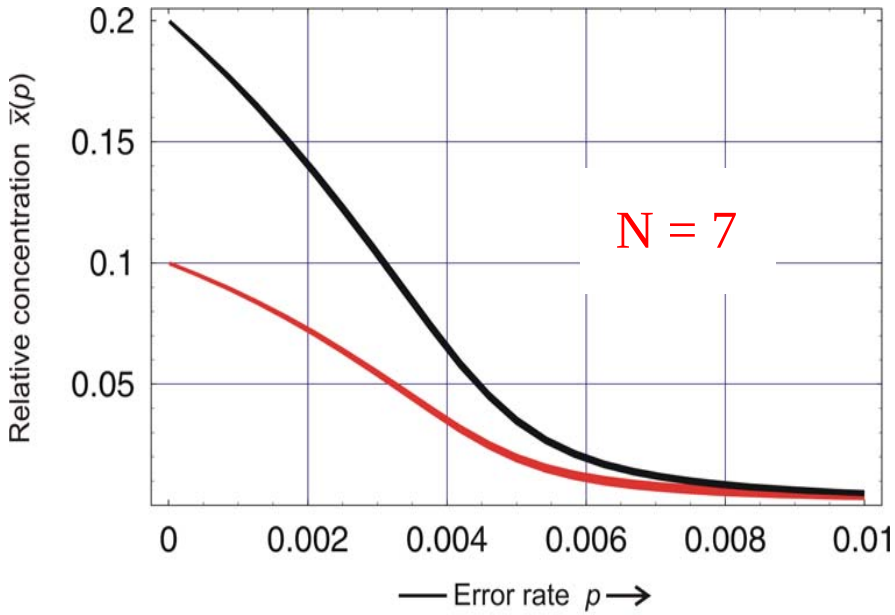


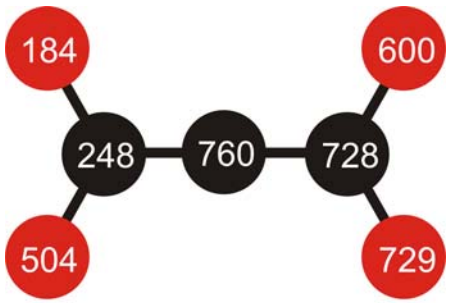


Neutral network

$\lambda = 0.10$, s = 229

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





Neutral network

$\lambda = 0.10,\ s = 229$

Perturbation matrix W

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

Eigenvalues of W

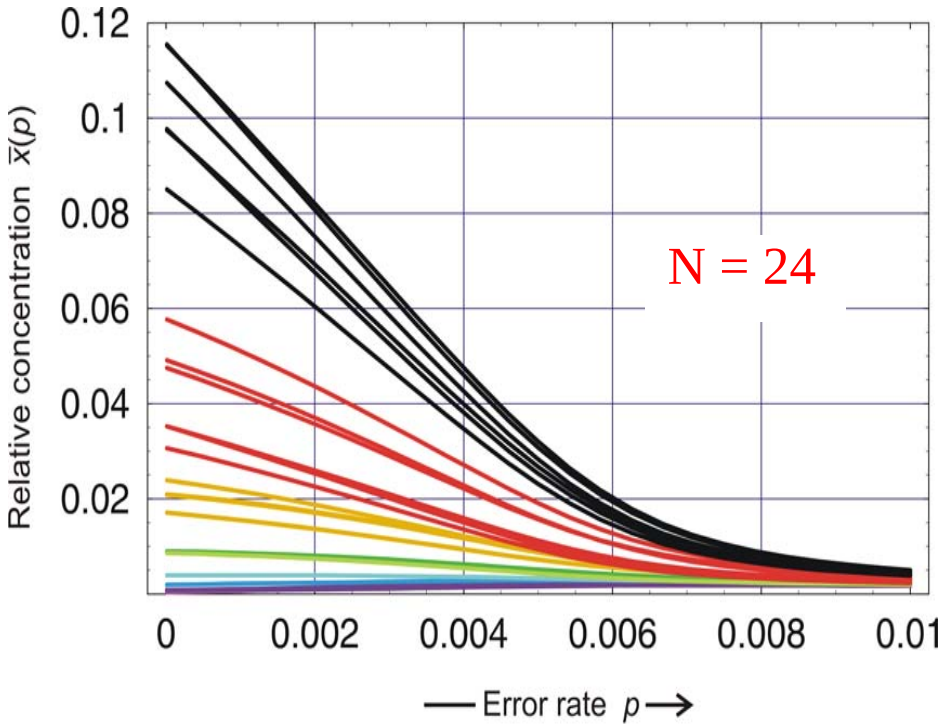
$$\lambda_0 = f + 2\varepsilon ,$$

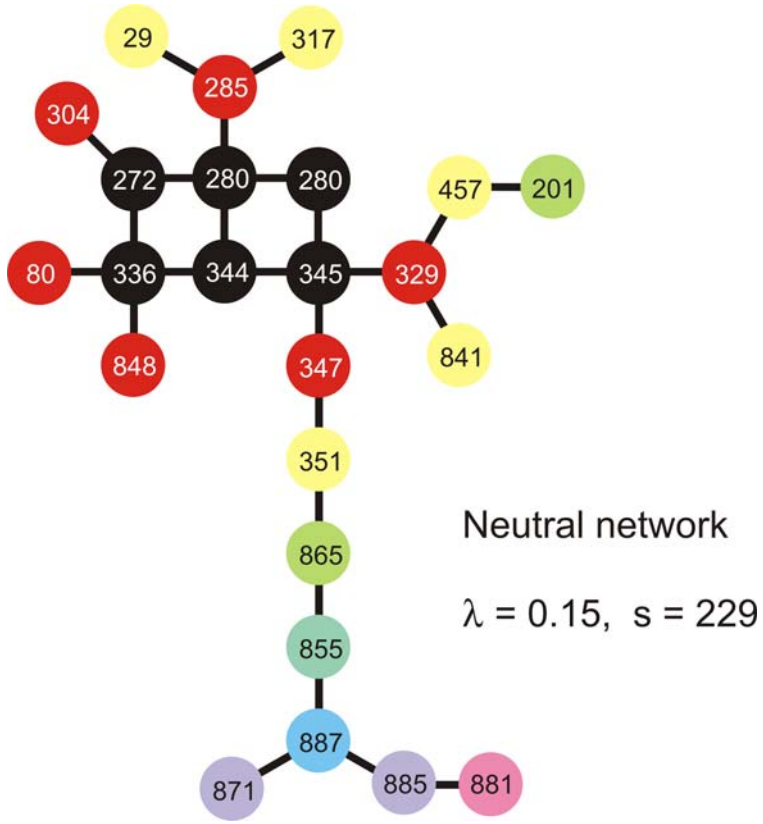
$$\lambda_1 = f + \sqrt{2}\varepsilon ,$$
$$\lambda_{2,3,4} = f ,$$
$$\lambda_5 = f - \sqrt{2}\varepsilon ,$$
$$\lambda_6 = f - 2\varepsilon .$$

Largest eigenvector of W

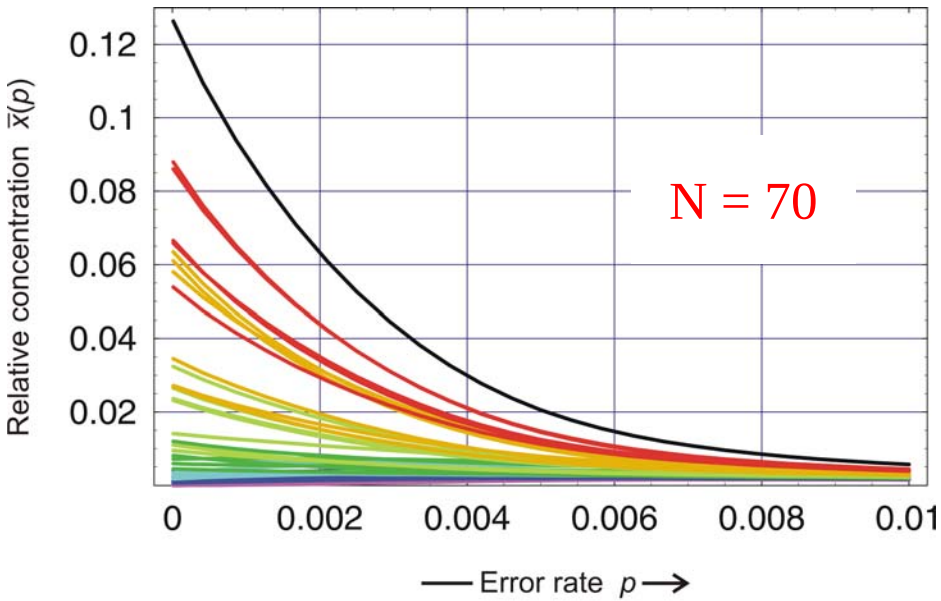
$$\xi_0 = (0.1, 0.1, 0.2, 0.2, 0.2, 0.1, 0.1) .$$

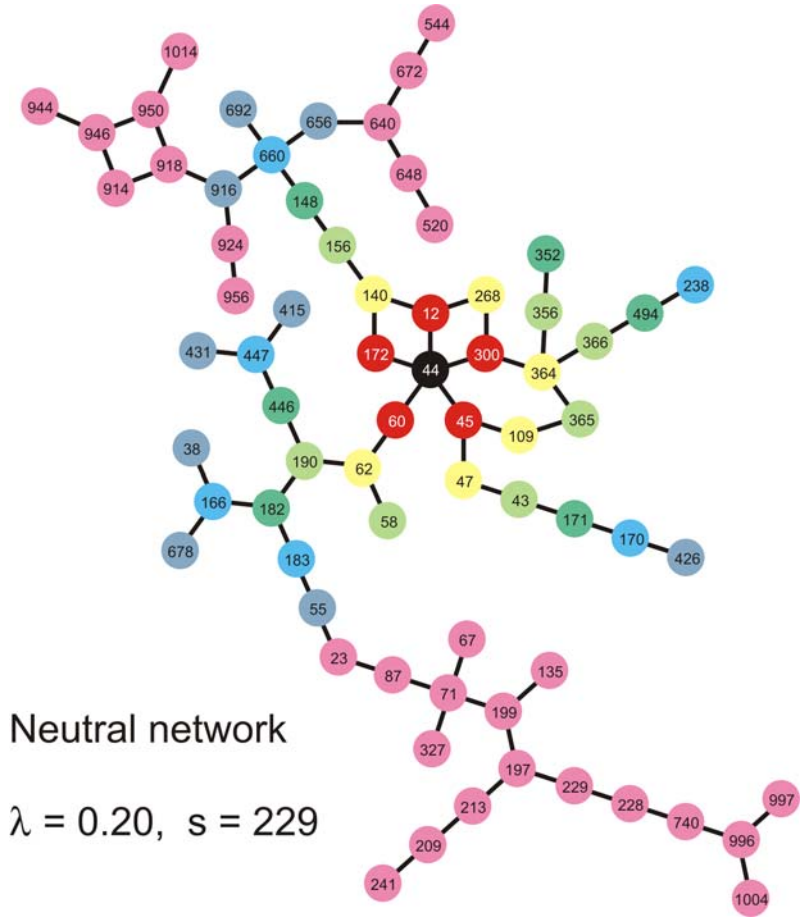
Neutral networks with increasing λ: λ = 0.10, s = 229





Neutral networks with increasing λ: λ = 0.15, s = 229





Neutral networks with increasing λ: λ = 0.20, s = 229

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