Neutral evolution – Inevitable and useful

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Shanghai, 12.09.2009

Web-Page for further information:

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

What is neutrality ?

Selective neutrality =
= several genotypes having the same fitness.

Structural neutrality =
= several genotypes forming molecules with the same structure.

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.

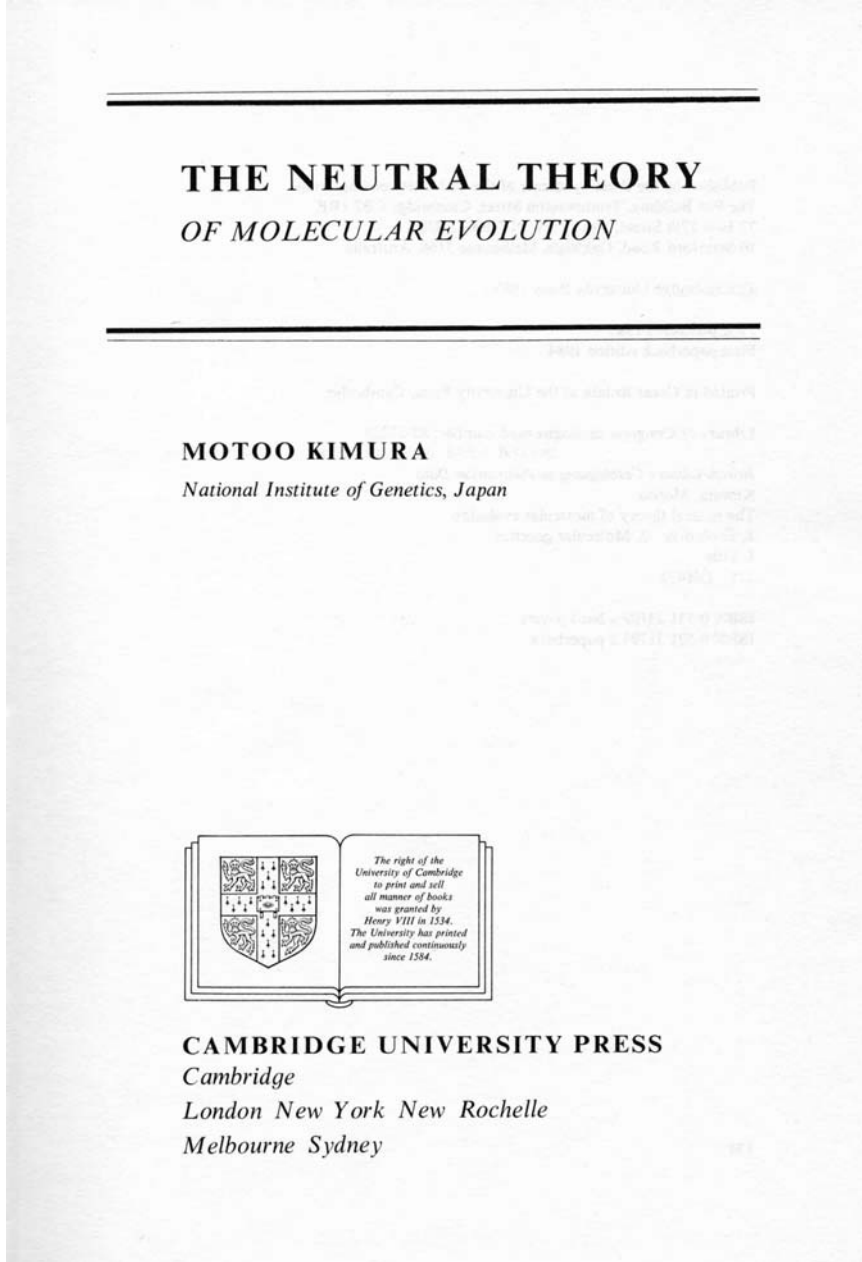
Charles Darwin. *The Origin of Species*. Sixth edition. John Murray. London: 1872

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.

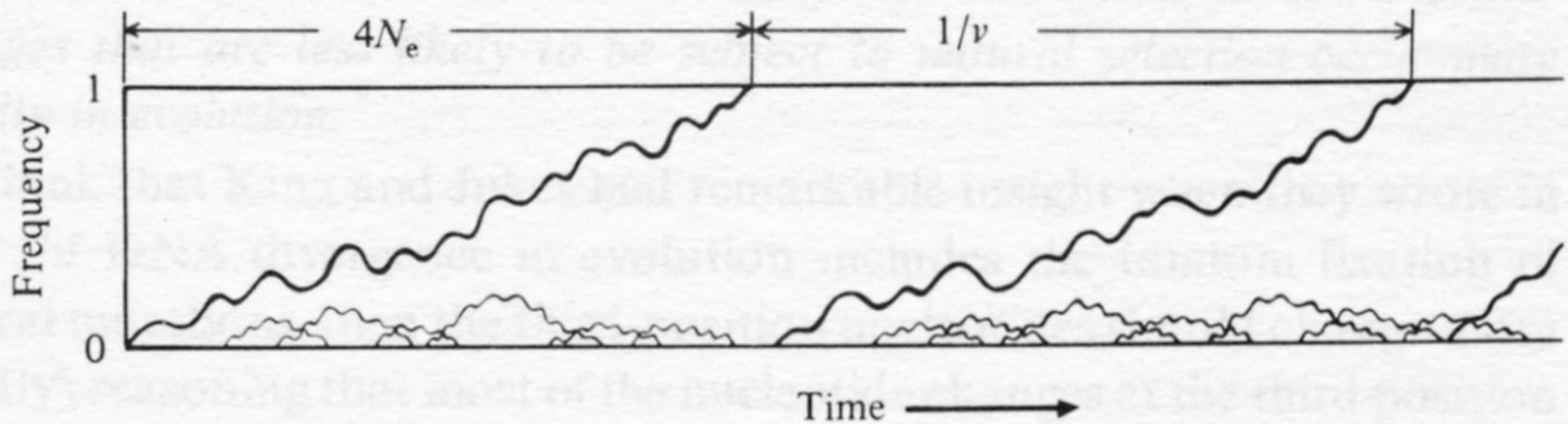


THE NEUTRAL THEORY
OF MOLECULAR EVOLUTION

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.

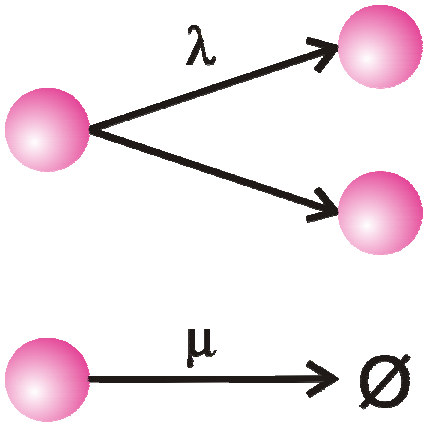


The average time of replacement of a dominant genotype in a population is the reciprocal mutation rate, 1/ν, and therefore independent of population size.

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

$$P_{n,m}(\mathrm{t}) = \mathrm{Prob}\left\{X(\mathrm{t}) = \mathrm{n} \mid X(0) = \mathrm{m}\right\}$$

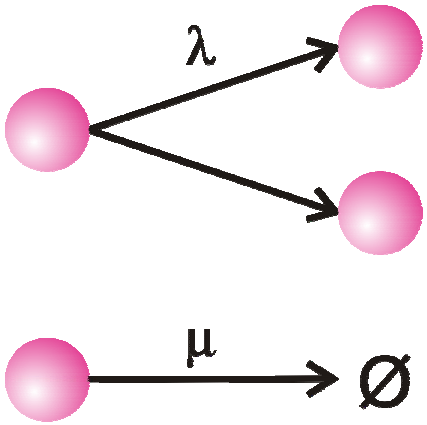
$$\frac{\mathrm{d}P_n}{\mathrm{dt}} = \lambda P_{n-1} + \mu P_{n+1} - (\lambda+\mu) P_n \; ; \quad P_n(0) = \delta_{n,m}$$



Neutral evolution as a linear birth-and-death process

$$P_{n,m}(t) = \text{Prob}\{X(t) = n \mid X(0) = m\}$$

$$\frac{dP_n}{dt} = \lambda P_{n-1} + \mu P_{n+1} - (\lambda+\mu) P_n \; ; \quad P_n(0) = \delta_{n,m}$$



$$\mu = \lambda$$

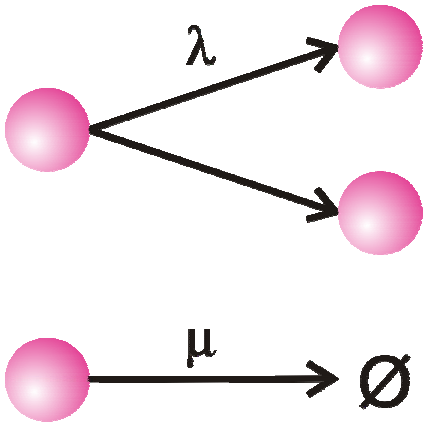
$$P_{n,m}(t) = \left(\frac{\lambda t}{1+\lambda t}\right)^{m+n} \sum_{k=0}^{(m,n)} \binom{m}{k} \binom{m+n-k-1}{n-k} \left(\frac{1 - \lambda^2 t^2}{\lambda^2 t^2}\right)^k$$

$$\langle n \rangle = m \quad \text{and} \quad \text{var}(n) = 2m\lambda t$$

Neutral evolution as a linear birth-and-death process

$$P_{n,m}(\mathrm{t}) = \mathrm{Prob}\left\{X(\mathrm{t}) = \mathrm{n} \mid X(0) = \mathrm{m}\right\}$$

$$\frac{dP_n}{dt} = \lambda P_{n-1} + \mu P_{n+1} - (\lambda+\mu) P_n \; ; \quad P_n(0) = \delta_{n,m}$$



$$\mu = \lambda$$

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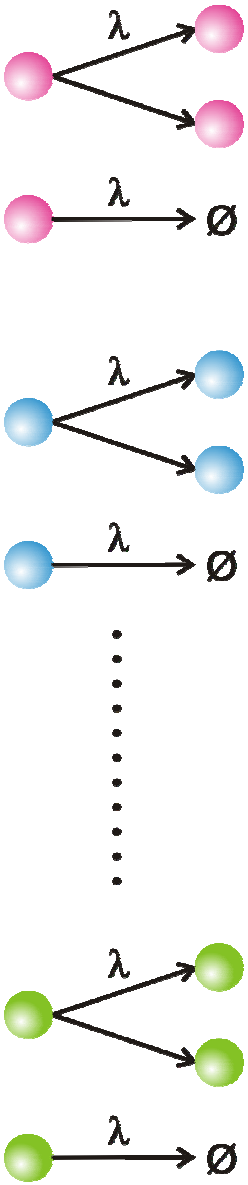
$$\langle n \rangle = m \quad \text{and} \quad \mathrm{var}(n) = 2m\lambda\mathrm{t}$$

$$P_{0,m}(\mathrm{t}) = \left(\frac{\lambda\mathrm{t}}{1+\lambda\mathrm{t}}\right)^m \quad \text{and hence} \quad \lim_{\mathrm{t}\to\infty} P_{0,m}(\mathrm{t}) = 1$$

Neutral evolution as a linear birth-and-death process

$$P_{0,1}(t) = P_0 = \frac{\lambda\mathrm{t}}{1+\lambda\mathrm{t}}$$



n different types, T_k ... time up to extinction of $n - k$ types

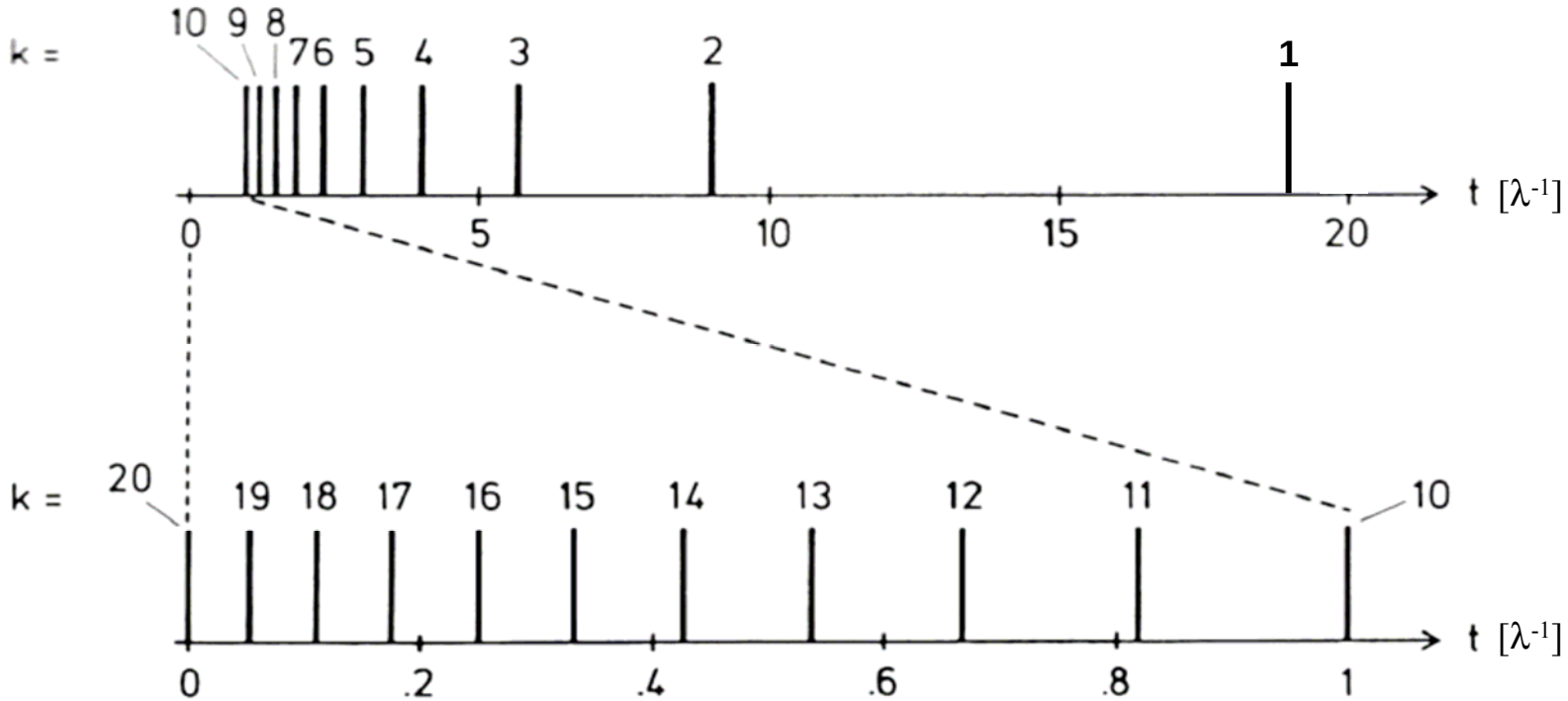
$$H_k(\mathrm{t}) = \mathrm{Prob}\{T_k < \mathrm{t}\}$$

$$H_0(\mathrm{t}) = P_{0,0,\ldots,0} = P_0^{(1)} \cdot P_0^{(2)} \cdot \ldots \cdot P_0^{(n)} = \left(\frac{\lambda \mathrm{t}}{1+\lambda \mathrm{t}}\right)^n$$

$$H_1(\mathrm{t}) = P_{x_1 \neq 0,0,\ldots,0} + P_{0,x_2 \neq 0,\ldots,0} + \ldots + P_{0,0,\ldots,x_n \neq 0} + H_0 \quad \textit{with} \quad P_{x \neq 0} = 1 - P_0 = \frac{1}{1+\lambda \mathrm{t}}$$

$$H_k(\mathrm{t}) = \sum_{j=0}^{k} \binom{n}{j} \frac{(\lambda \mathrm{t})^{n-j}}{(1+\lambda \mathrm{t})^n} \quad \text{and} \quad \langle T_k \rangle = \frac{n-k}{k \cdot \lambda}, \ \mathrm{var}(T_k) = \frac{n(n-k)}{k^2 (k-1) \cdot \lambda^2}$$

Neutral evolution as a linear birth-and-death process



The distribution of sequential extinction times, $< T_k >$, for $n = 20$

RANDOM SELECTION — A SIMPLE MODEL BASED ON LINEAR BIRTH AND DEATH PROCESSES†

■ P. SCHUSTER and K. SIGMUND‡
Institut für Theoretische Chemie und Strahlenchemie and
‡Institut für Mathematik,
Universität Wien,
A-1090 Wien, Austria

Linear birth and death processes are used to derive simple expressions for sequential extinction times and gene fixation probabilities in asexual populations.

$$\left\langle T_k \right\rangle = \frac{n-k}{k}\,\lambda^{-1} \quad \text{and} \quad \left\langle T_0 \right\rangle = \infty$$

1. Origins of neutrality
2. RNA replication and quasispecies
3. Selection on realistic landscapes
4. Consequences of neutrality
5. Evolutionary optimization *in silico*

1. **Origins of neutrality**
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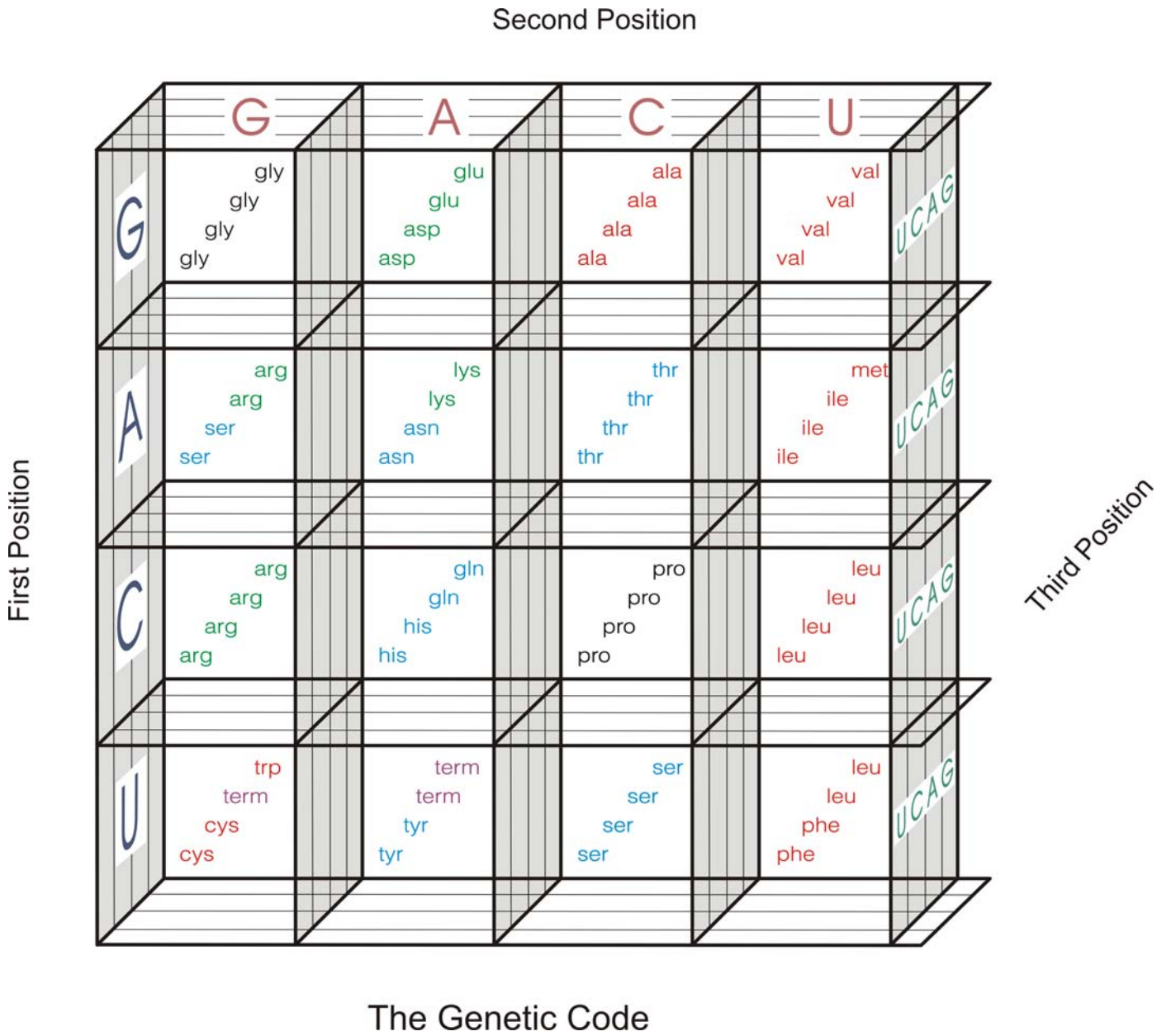
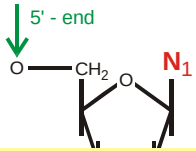
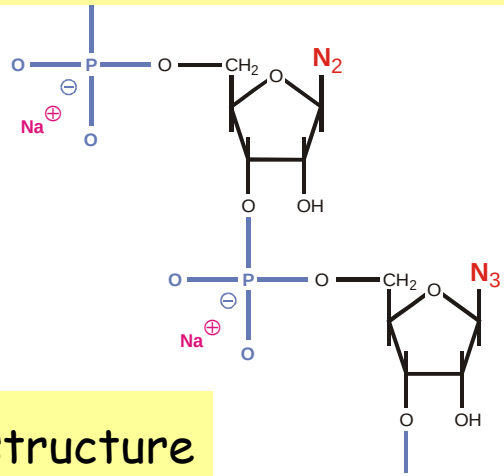
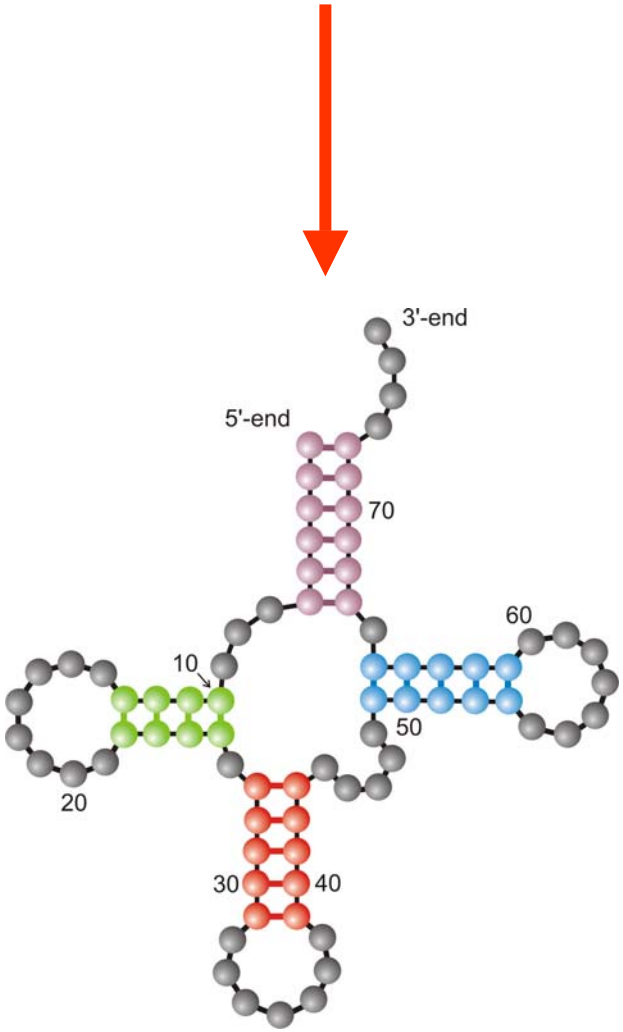


Redundancy of the genetic code as a source of neutrality

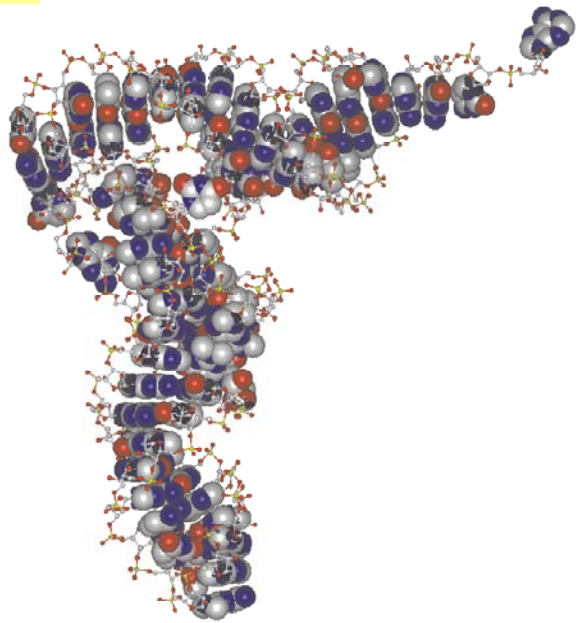


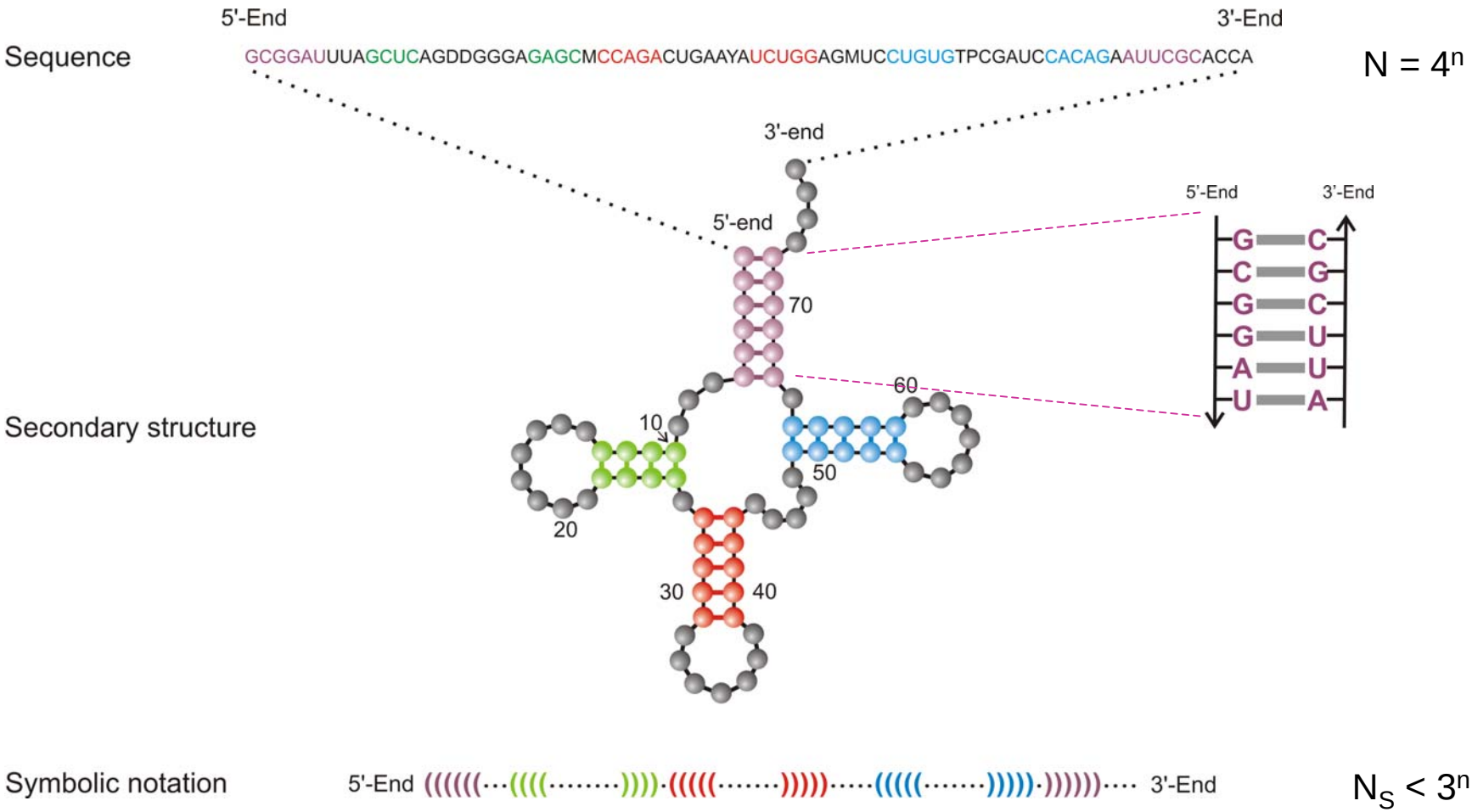
5'-end GCGGAUUUAGCUCAGUUGGGAGAGCGCCAGACUGAAGAUCUGGAGGUCCUGUGUUCGAUCCACAGAAUUCGCACCA 3'-end





Definition of RNA structure

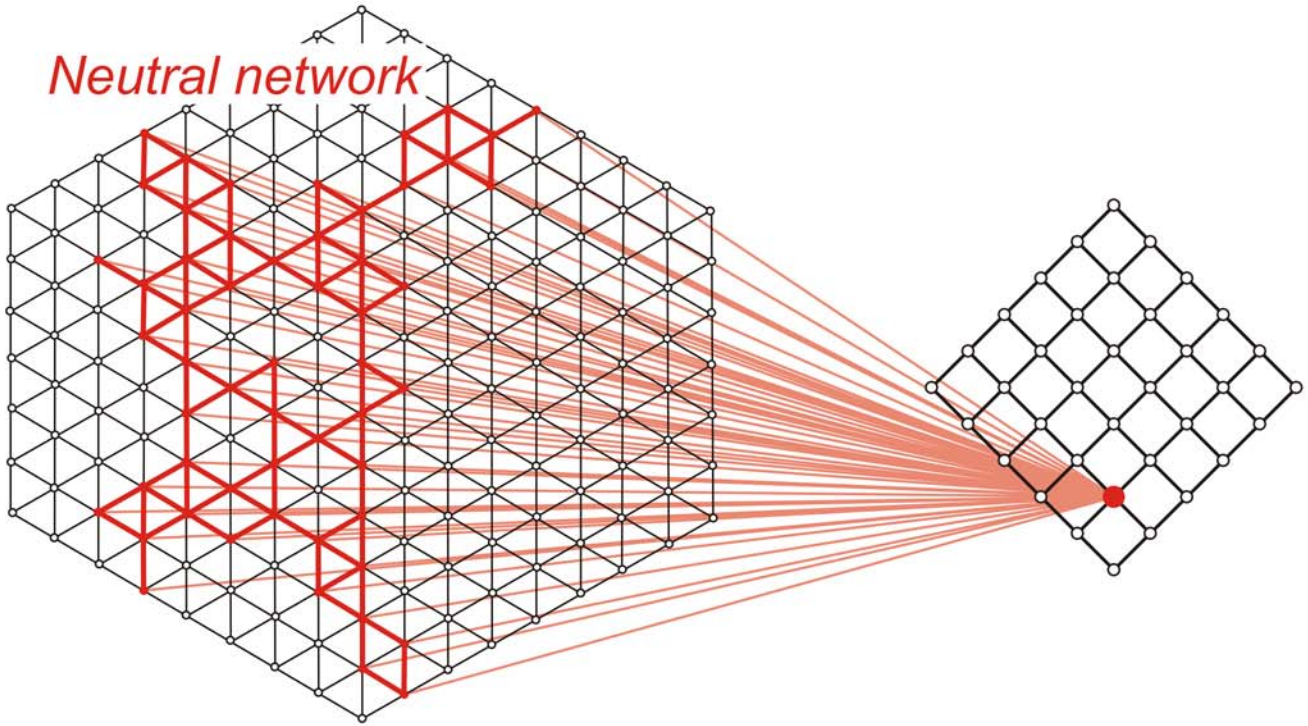




Criterion: Minimum free energy (mfe)

Rules: _ (_) _ ∈ {**AU**,**CG**,**GC**,**GU**,**UA**,**UG**}

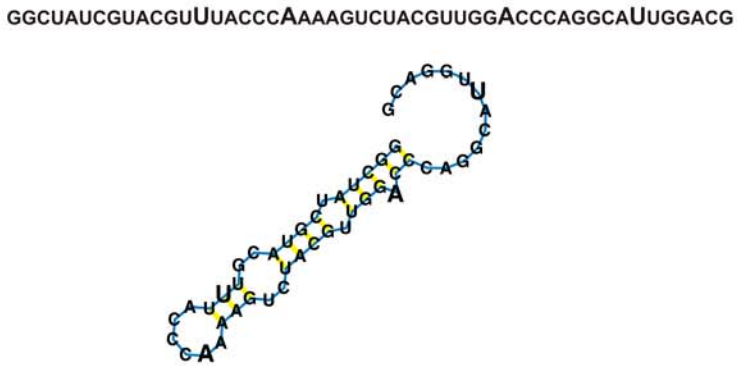
A symbolic notation of RNA secondary structure that is equivalent to the conventional graphs



Sequence space

Structure space

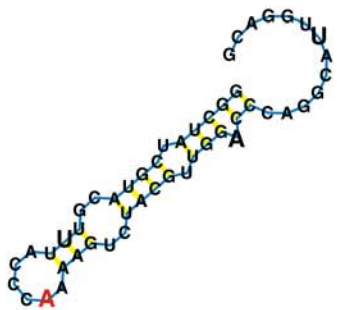
many genotypes ⇒ one phenotype



One error neighborhood – Surrounding of an RNA molecule of chain length n=50 in sequence and shape space

GGCUAUCGUACGUUUACCCGAAAGUCUACGUUGGACCCAGGCAUUGGACG

GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG



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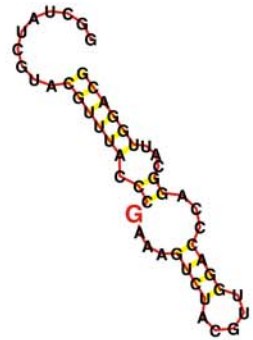
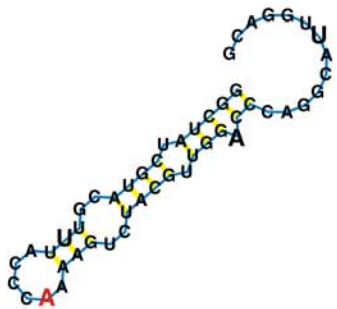


GGCUAUCGUACGUUUACCCGAAAGUCUACGUUGGACCCAGGCAUUGGACG

GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG



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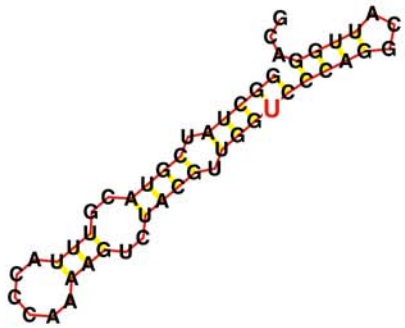
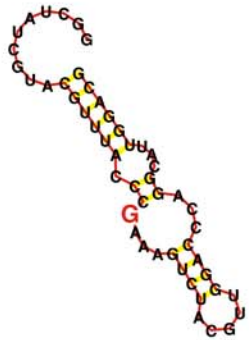
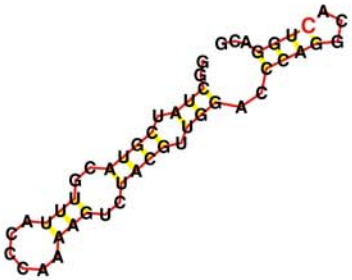


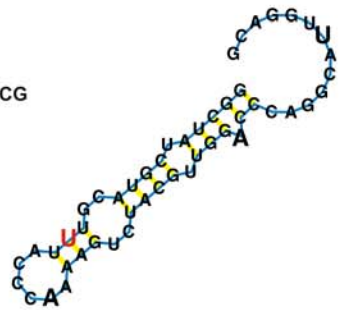
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGUCCCAGGCAUUGGACG



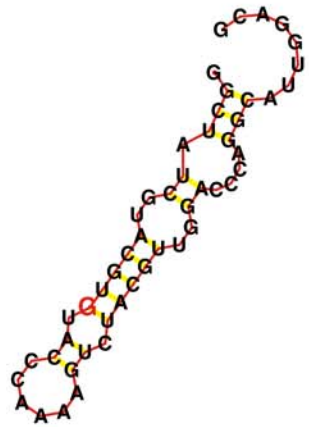
GGCUAUCGUACGUUUACCCGAAAGUCUACGUUGGACCCAGGCAUUGGACG

GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCACUGGACG





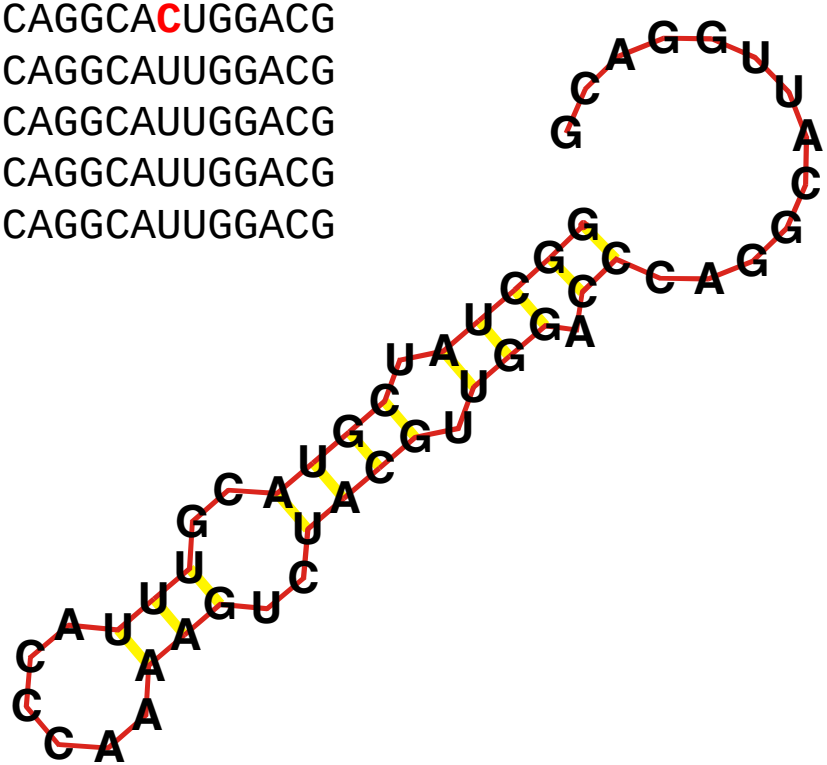
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG



GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG

One error neighborhood – Surrounding of an RNA molecule of chain length n=50 in sequence and shape space

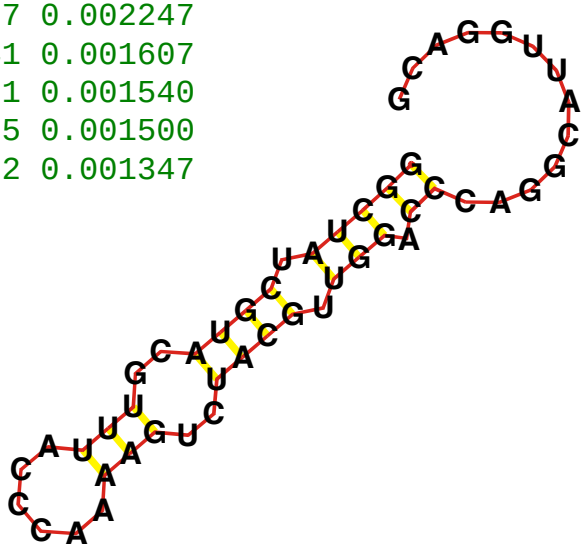
GGCUAUCGUA**U**GUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUU**A**GACG
GGCUAUCGUACGUUUAC**U**CAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUAUCGUACG**C**UUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGC**C**AUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUAUCGUACGU**G**UACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUA**A**CGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCC**U**GGCAUUGGACG
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCA**C**UGGACG
GGCUAUCGUACGUUUACCCAAAAGUCUACGUUGG**U**CCCAGGCAUUGGACG
GGCUA**G**CGUACGUUUACCCAAAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUAUCGUACGUUUACCC**G**AAAGUCUACGUUGGACCCAGGCAUUGGACG
GGCUAUCGUACGUUUACCCAAAAG**C**CUACGUUGGACCCAGGCAUUGGACG



One error neighborhood – Surrounding of an RNA molecule of chain length n=50 in sequence and shape space

	Number	Mean Value	Variance	Std.Dev.
Total Hamming Distance:	150000	11.647973	23.140715	4.810480
Nonzero Hamming Distance:	99875	16.949991	30.757651	5.545958
Degree of Neutrality:	50125	**0.334167**	0.006961	**0.083434**
Number of Structures:	**1000**	**52.31**	85.30	**9.24**

1	(((((.((((..(((.....))))..)))))..))).).....	**50125**	**0.334167**
2	..(((.((((..(((.....))))..)))))..))).).....	**2856**	**0.019040**
3	((((((((((..(((.....))))..)))))))).)).....	**2799**	**0.018660**
4	(((((.((((..((((....))))..)))))..))).)).....	**2417**	**0.016113**
5	(((((.((((.((((.....))))).)))))..))).)).....	**2265**	**0.015100**
6	(((((.(((((.(((.....))).)))))..))).)).....	**2233**	**0.014887**
7	(((((..(((..(((.....)))..)))..)))..)).....	1442	0.009613
8	(((((.((((..((.....)).)))))..))).)).....	1081	0.007207
9	((((..((((..(((.....)))..)))))..)).)).....	1025	0.006833
10	(((((.((((..(((.....)))..)))))..))))).....	1003	0.006687
11	.((((.((((..(((.....)))..)))))..)))).....	963	0.006420
12	(((((.(((...(((.....)))...)))..))).)).....	860	0.005733
13	(((((.((((..(((.....)))..)))))..)).))).....	800	0.005333
14	(((((.((((...((.....))...)))))..))).)).....	548	0.003653
15	(((((.((((.............))))..))).)).....	362	0.002413
16	((.((.((((..(((.....)))..)))))..))..)).....	337	0.002247
17	(.(((.((((..(((.....)))..)))))..))).).....	241	0.001607
18	(((((.(((((((((.....))))))))).))).)).....	231	0.001540
19	((((..((((..(((.....)))..)))))...)))).....	225	0.001500
20	((....((((..(((.....)))..))))).....)).....	202	0.001347



Shadow – Surrounding of an RNA structure in shape space:
AUGC alphabet, chain length n=50

1. Origins of neutrality
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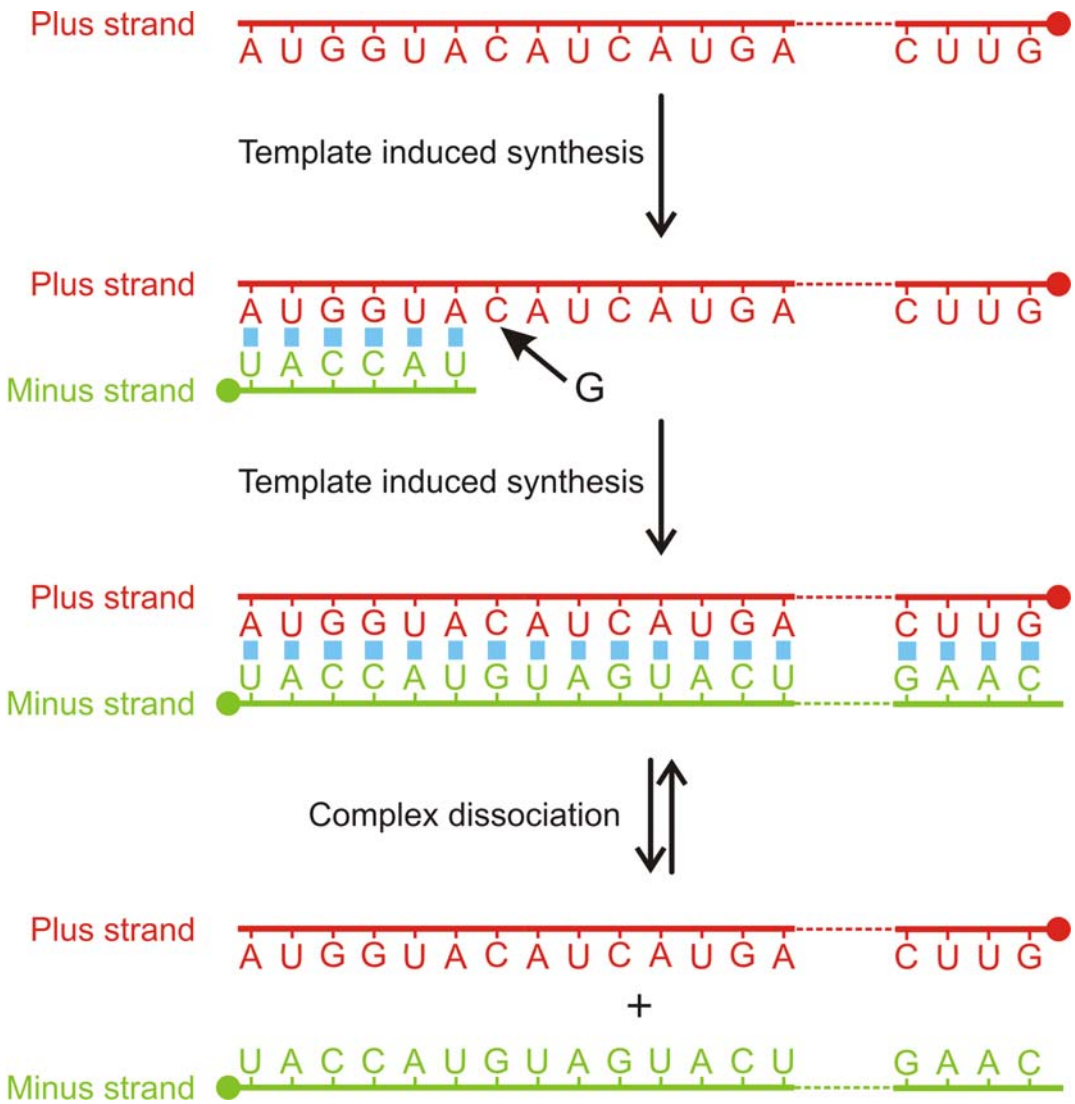
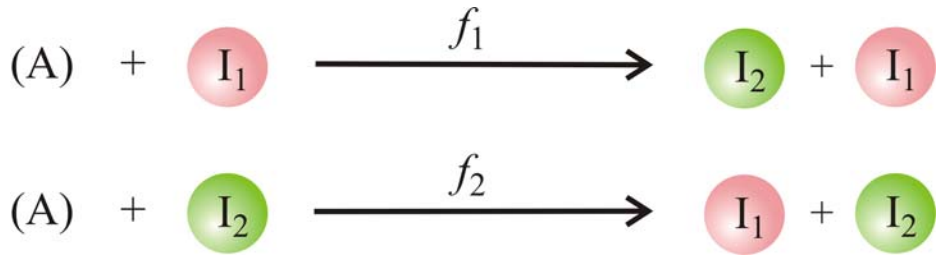


Complementary replication is the simplest copying mechanism of RNA.
Complementarity is determined by Watson-Crick base pairs:

G≡C and **A=U**



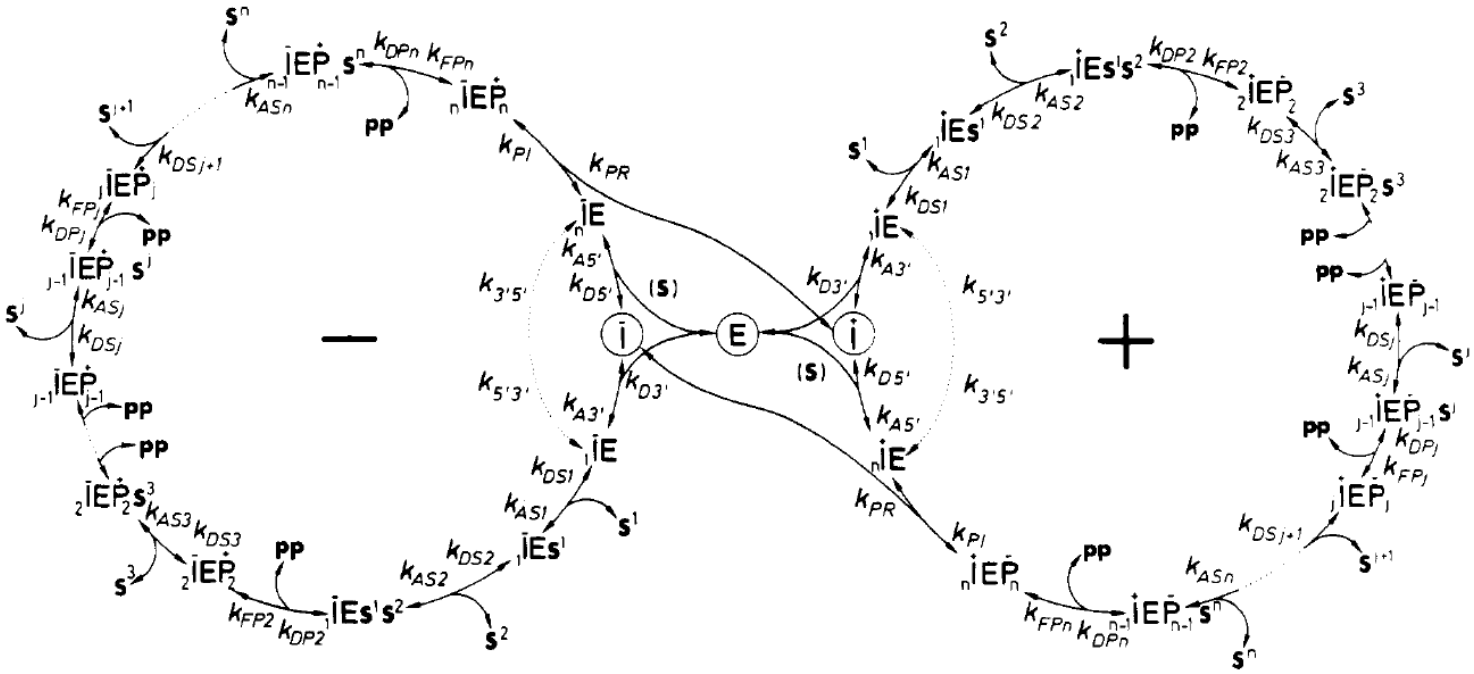
$$\frac{dx_1}{dt} = f_2\, x_2 \quad \text{and} \quad \frac{dx_2}{dt} = f_1\, x_1$$

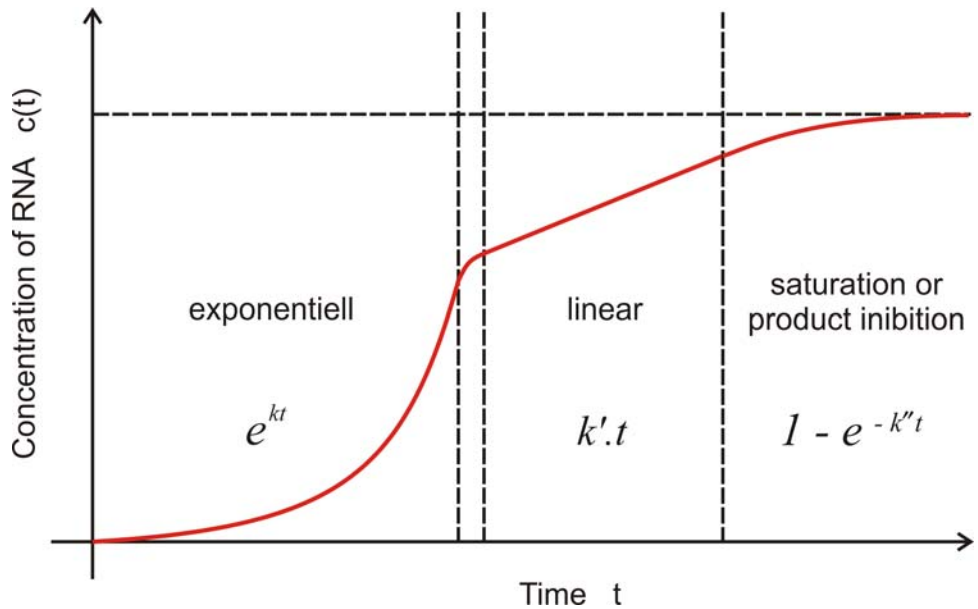
$$x_1 = \sqrt{f_2}\;\xi_1\,, \quad x_2 = \sqrt{f_1}\;\xi_2\,, \quad \zeta = \xi_1 + \xi_2\,, \quad \eta = \xi_1 - \xi_2\,, \quad f = \sqrt{f_1 f_2}$$

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

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

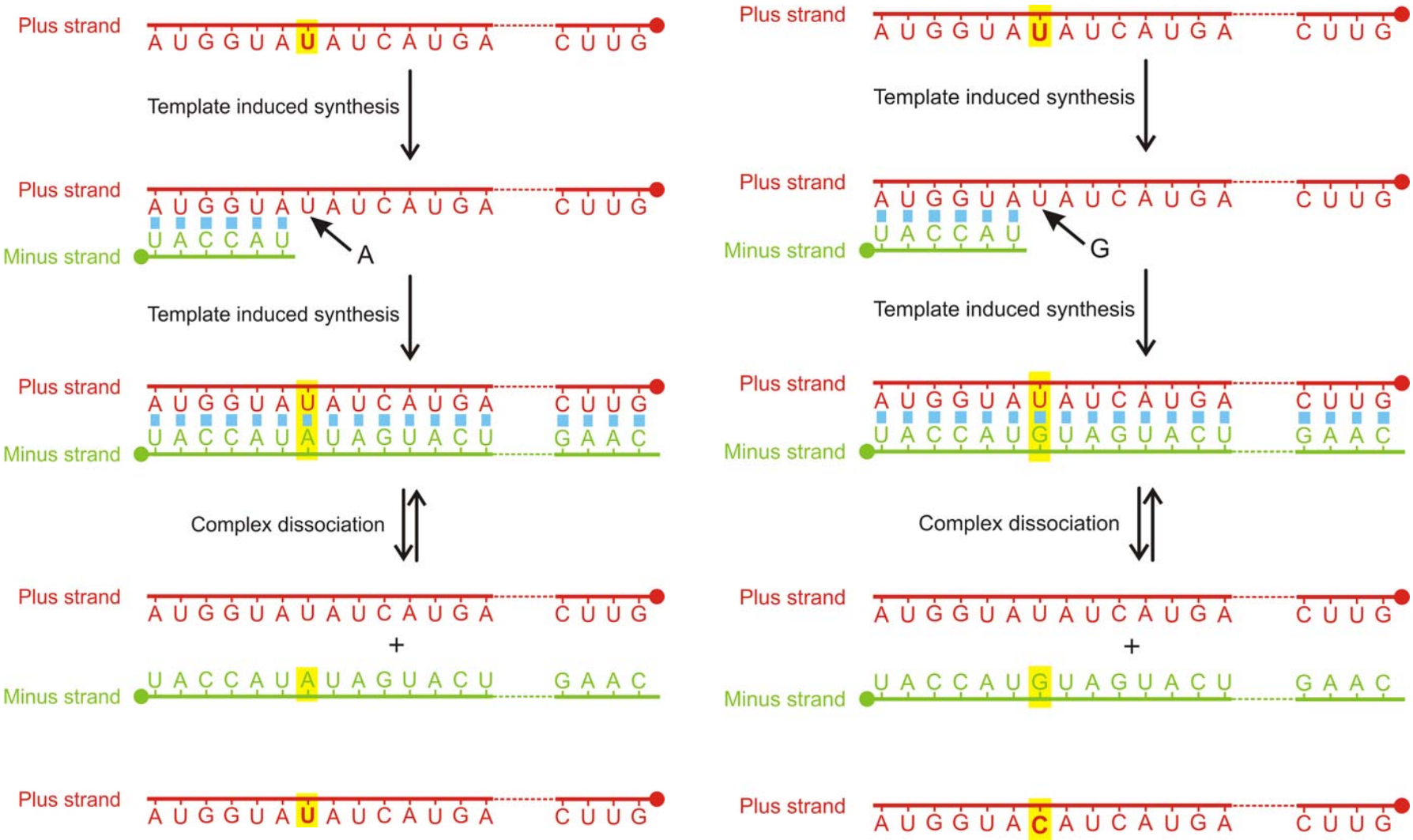
Complementary replication as the simplest molecular mechanism of reproduction



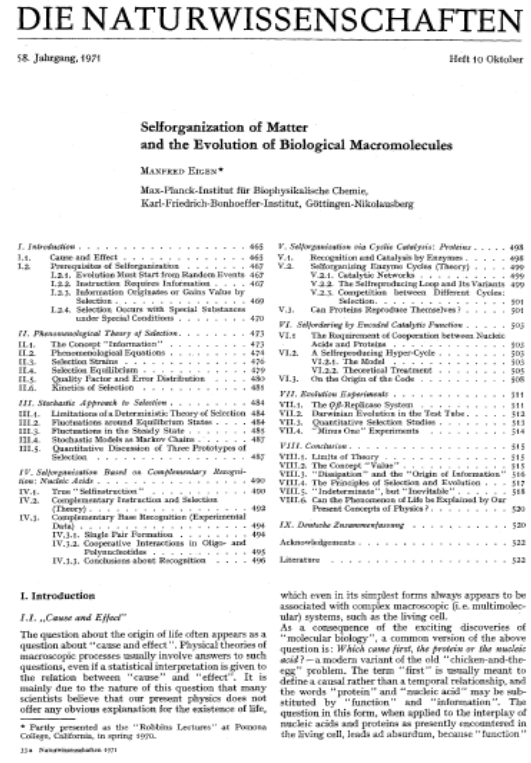


Kinetics of RNA replication

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



Replication and mutation are parallel chemical reactions.



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

I. Introduction

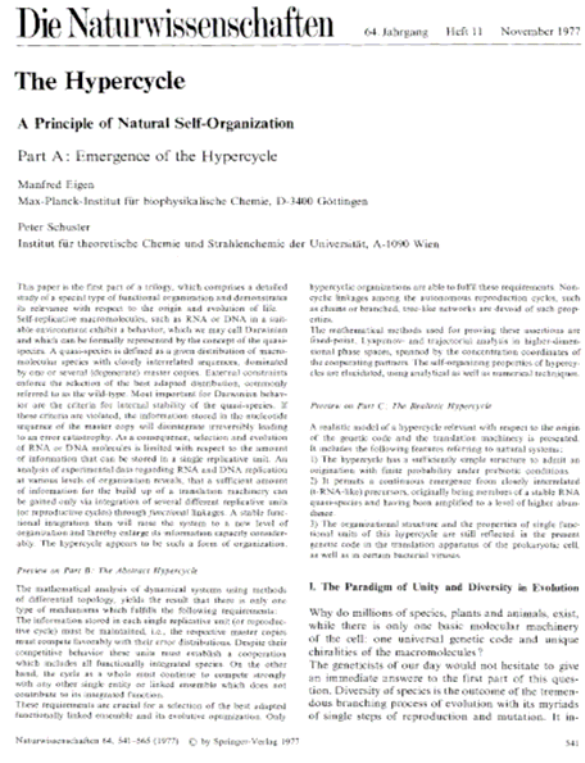
I.1. „Cause and Effect"

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-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"

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

1971



Die Naturwissenschaften 64. Jahrgang Heft 11 November 1977

The Hypercycle

A Principle of Natural Self-Organization

Part A: Emergence of the Hypercycle

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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-cyclic 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†

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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. Combinatory 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.

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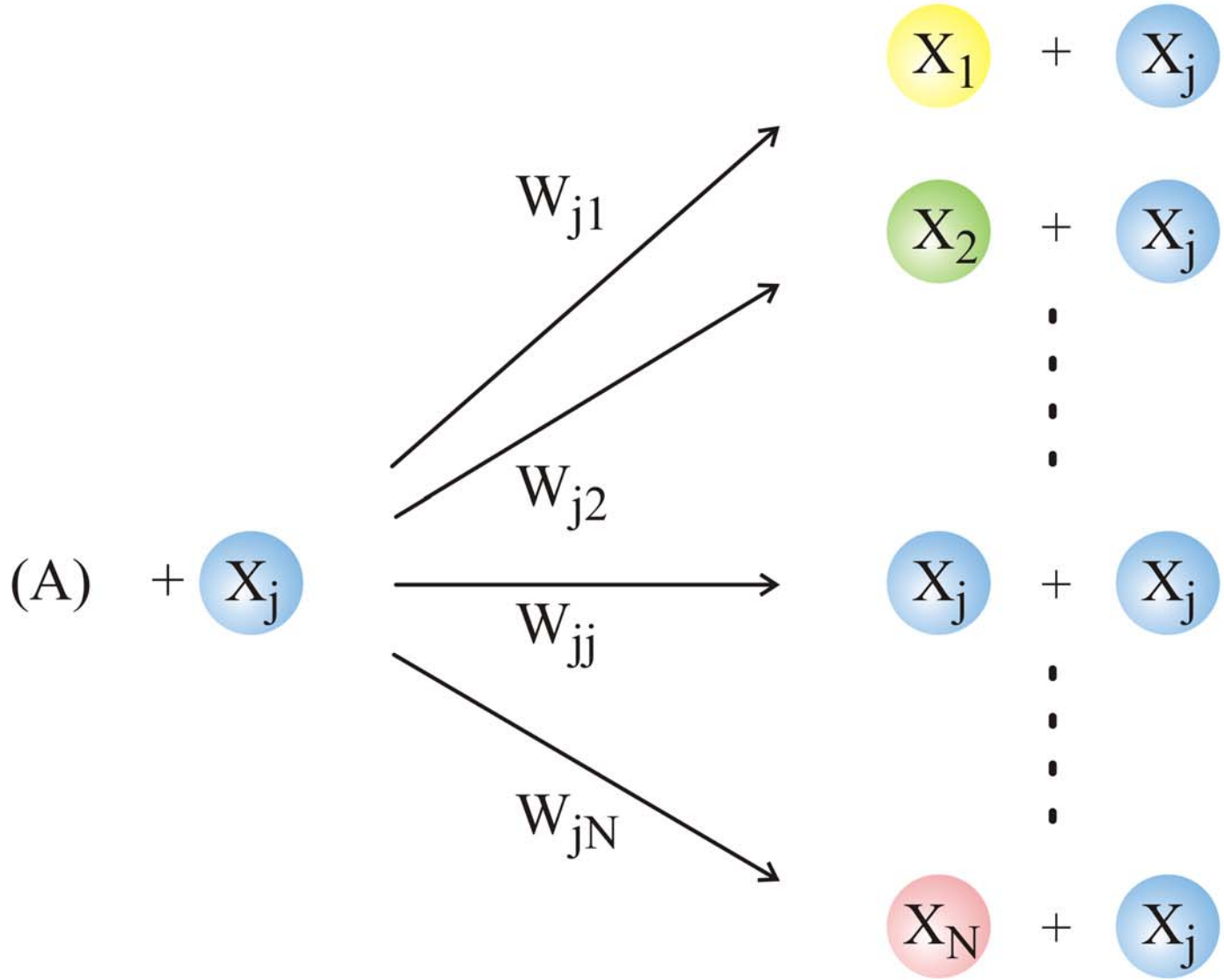
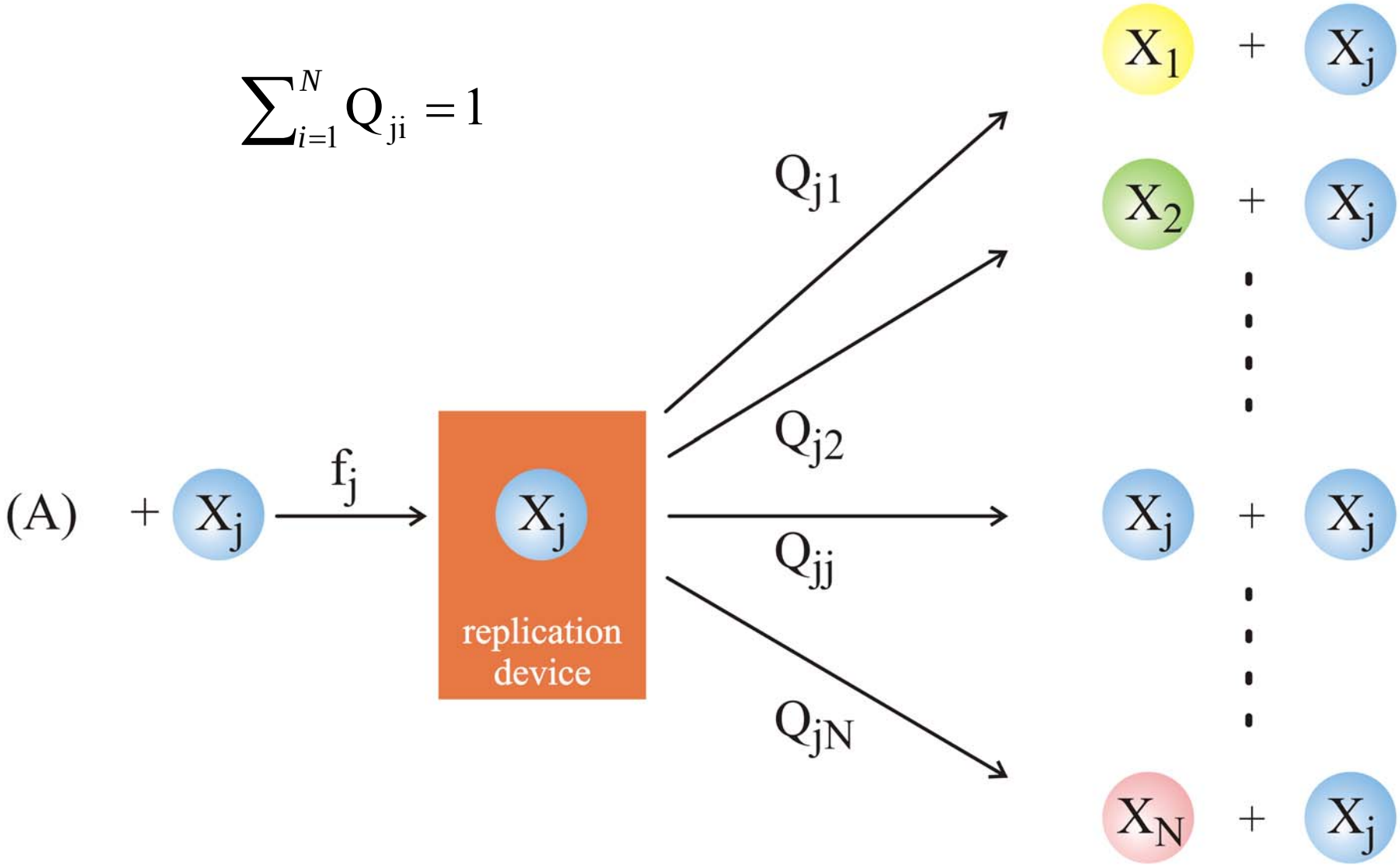
1988

Chemical kinetics of molecular evolution

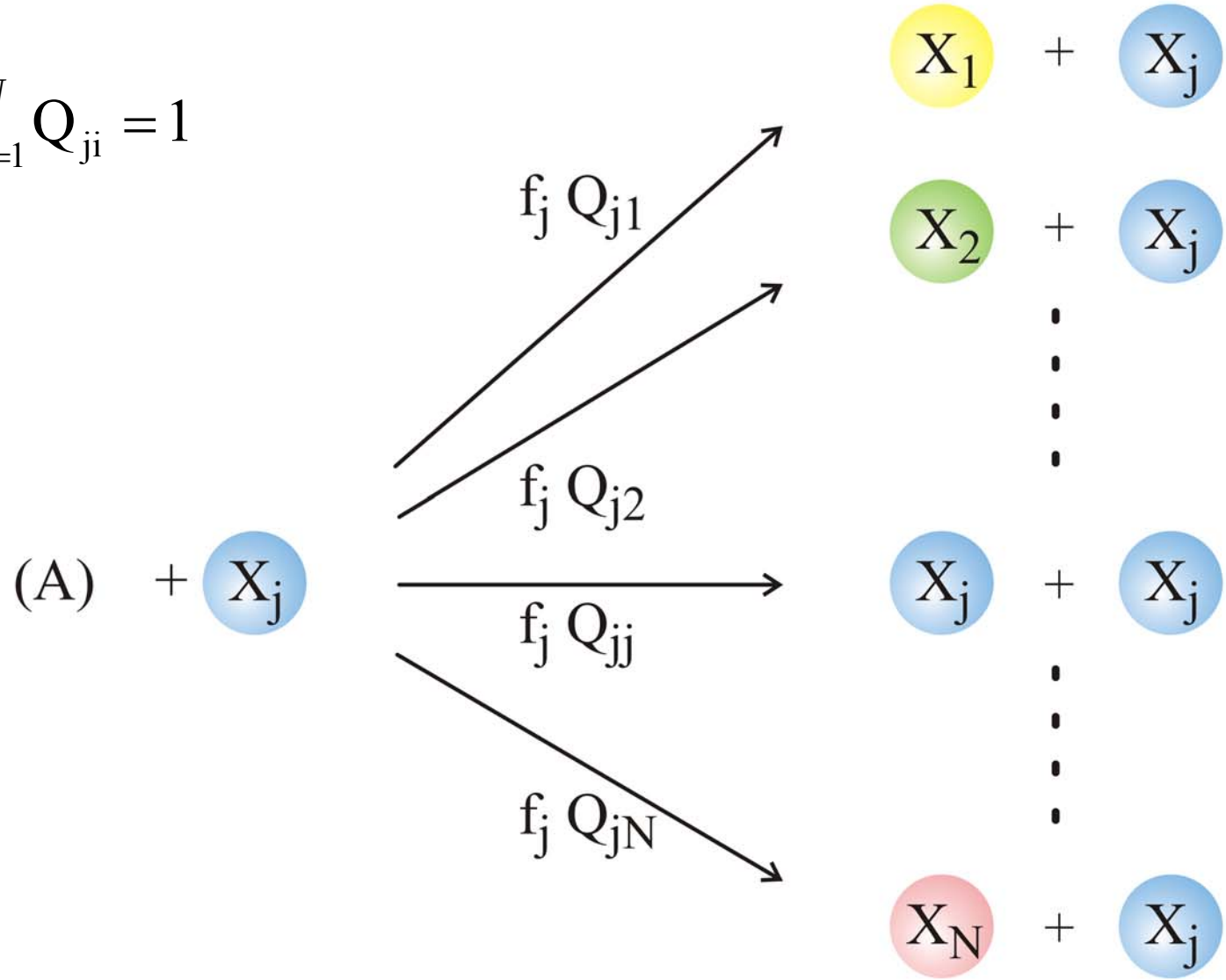


Chemical kinetics of replication and mutation as parallel reactions



Chemical kinetics of replication and mutation as parallel reactions

$$\sum_{i=1}^{N} Q_{ji} = 1$$



Chemical kinetics of replication and mutation as parallel reactions

Decomposition of matrix W

$$\mathrm{W} = \begin{pmatrix} w_{11} & w_{12} & \dots & w_{1n} \\ w_{21} & w_{22} & \dots & w_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ w_{n1} & w_{n2} & \dots & w_{nn} \end{pmatrix} = \mathrm{Q} \cdot \mathrm{F} \quad \text{with}$$

$$\mathrm{Q} = \begin{pmatrix} Q_{11} & Q_{12} & \dots & Q_{1n} \\ Q_{21} & Q_{22} & \dots & Q_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ Q_{n1} & Q_{n2} & \dots & Q_{nn} \end{pmatrix} \quad \text{and} \quad \mathrm{F} = \begin{pmatrix} f_1 & 0 & \dots & 0 \\ 0 & f_2 & \dots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \dots & f_n \end{pmatrix}$$

Factorization of the value matrix W separates mutation and fitness effects.

Mutation-selection equation: $[\mathrm{I}_i] = x_i \geq 0,\ f_i \geq 0,\ Q_{ij} \geq 0$

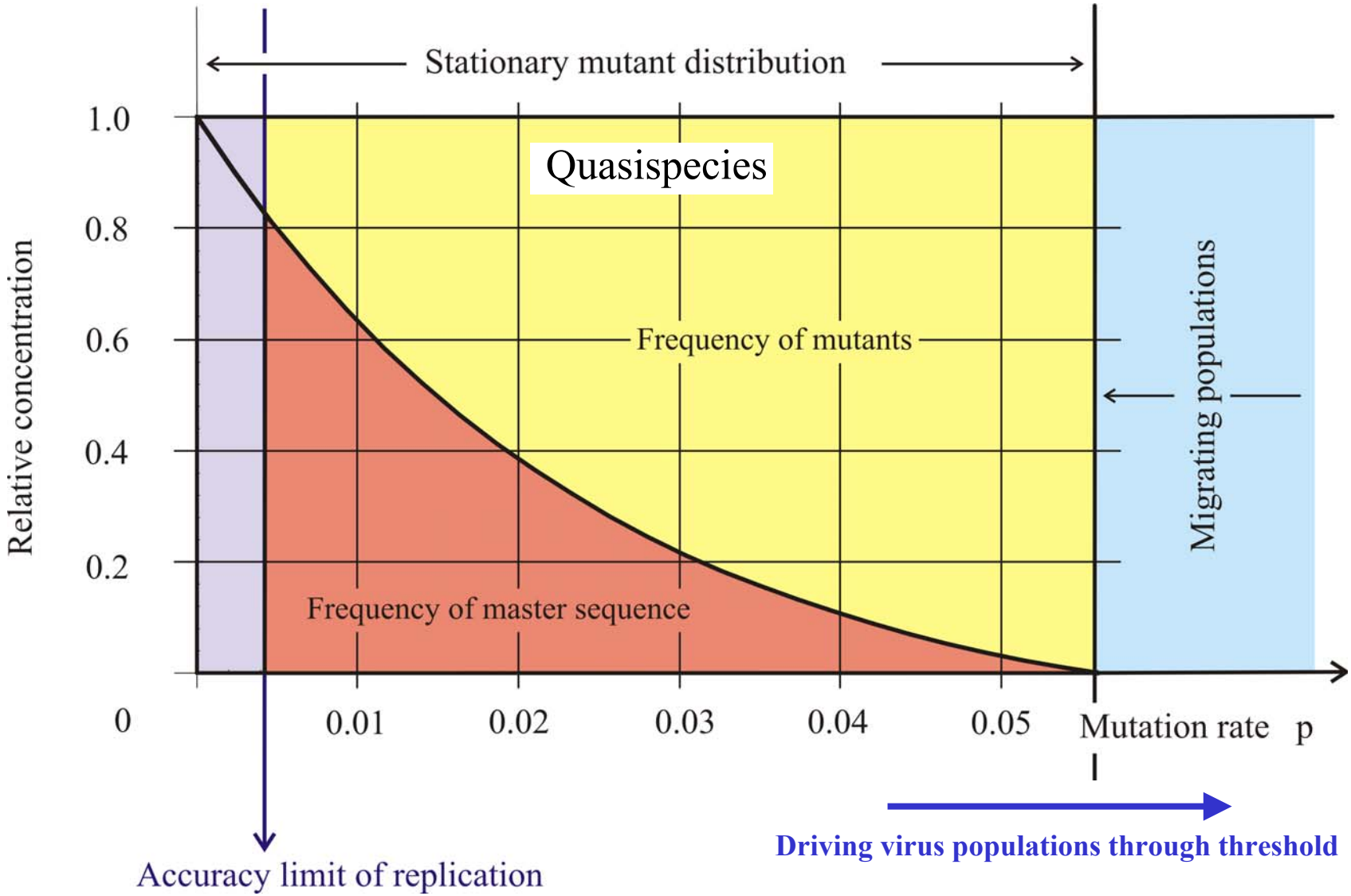
$$\frac{dx_i}{dt} = \sum_{j=1}^{n} Q_{ij}\ f_j\ x_j - x_i\,\phi, \quad i = 1,2,\cdots,n; \quad \sum_{i=1}^{n} x_i = 1; \quad \phi = \sum_{j=1}^{n} f_j\, x_j = \overline{f}$$

solutions are obtained after integrating factor transformation by means of an eigenvalue problem

$$x_i(t) = \frac{\sum_{k=0}^{n-1} \ell_{ik} \cdot c_k(0) \cdot \exp(\lambda_k t)}{\sum_{j=1}^{n} \sum_{k=0}^{n-1} \ell_{jk} \cdot c_k(0) \cdot \exp(\lambda_k t)}; \quad i = 1,2,\cdots,n; \quad c_k(0) = \sum_{i=1}^{n} h_{ki}\ x_i(0)$$

$$W \div \left\{ f_i Q_{ij};\ i, j = 1,2,\cdots,n \right\};\ L = \left\{ \ell_{ij};\ i, j = 1,2,\cdots,n \right\};\ L^{-1} = H = \left\{ h_{ij};\ i, j = 1,2,\cdots,n \right\}$$

$$L^{-1} \cdot W \cdot L \quad = \quad \Lambda \quad = \quad \left\{ \lambda_k; k = 0, 1, \cdots, n-1 \right\}$$



The error threshold in replication

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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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DOI: 10.1002/anie.200701369

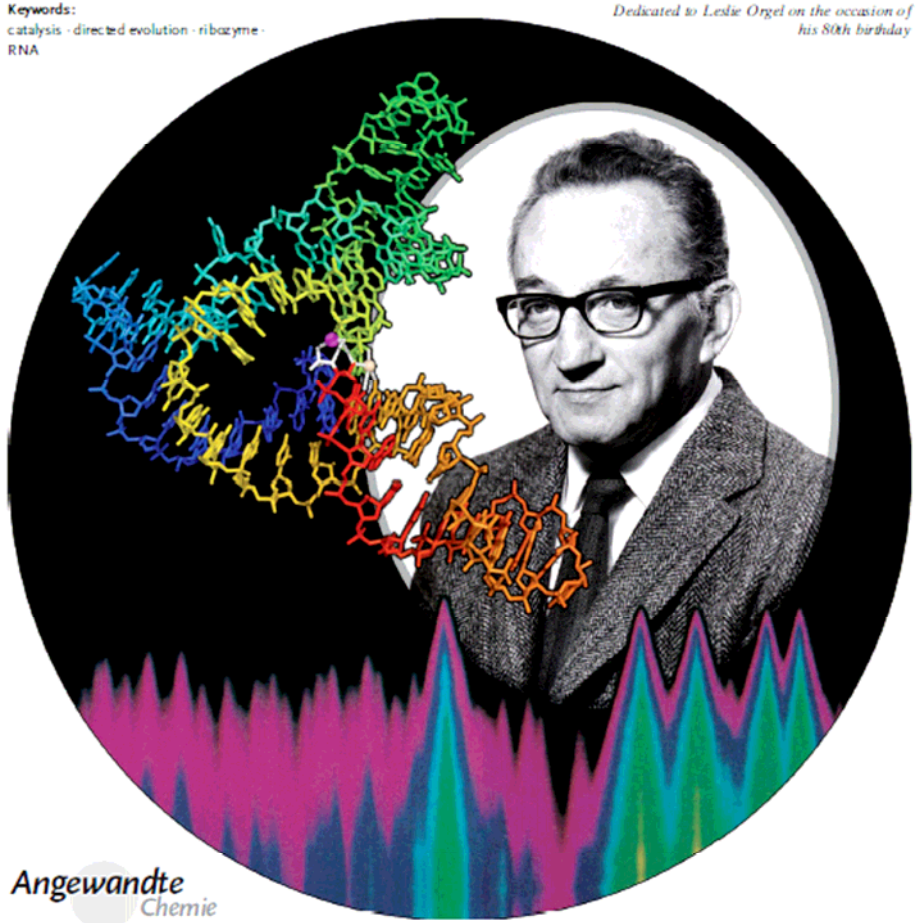
Molecular Evolution

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



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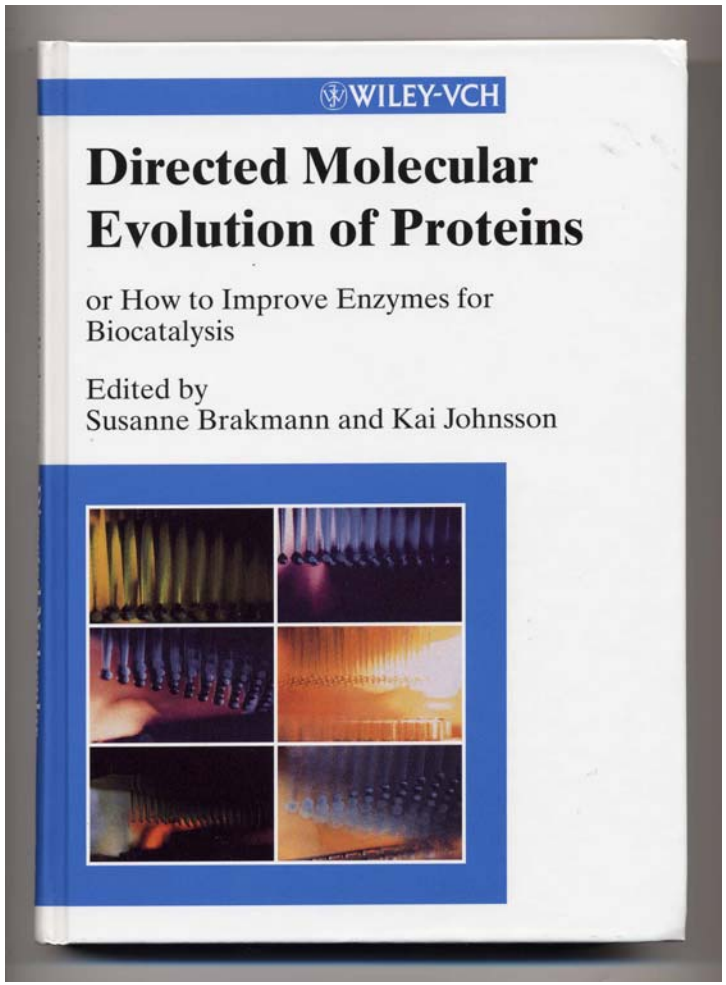

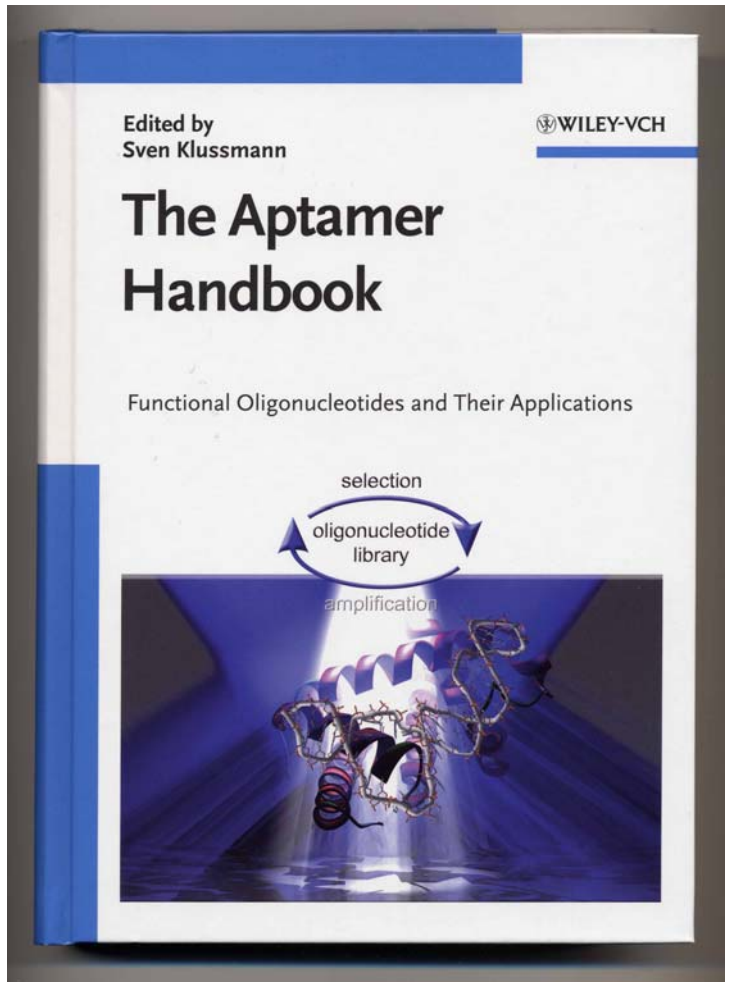

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Artificial evolution in biotechnology and pharmacology

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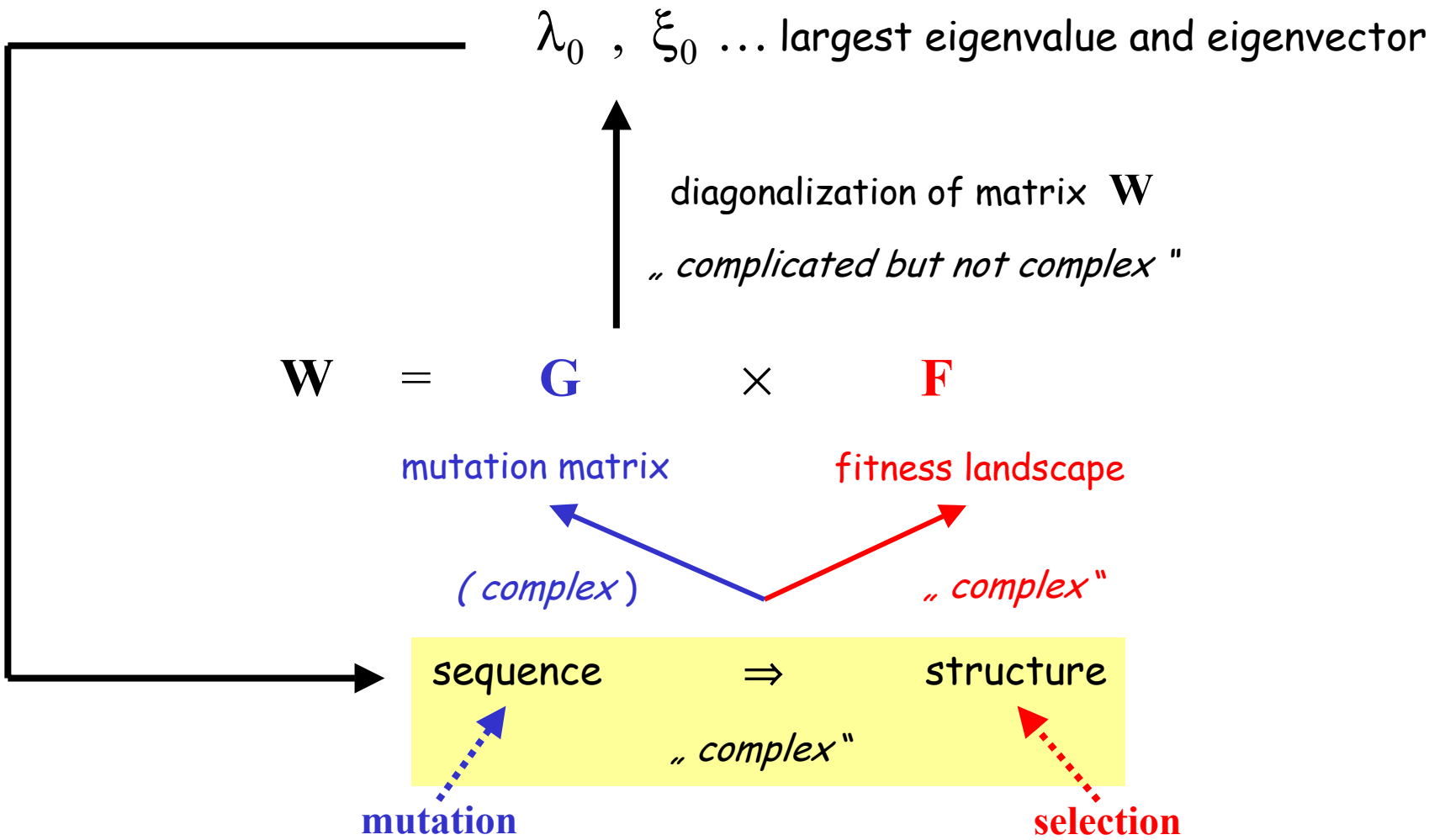
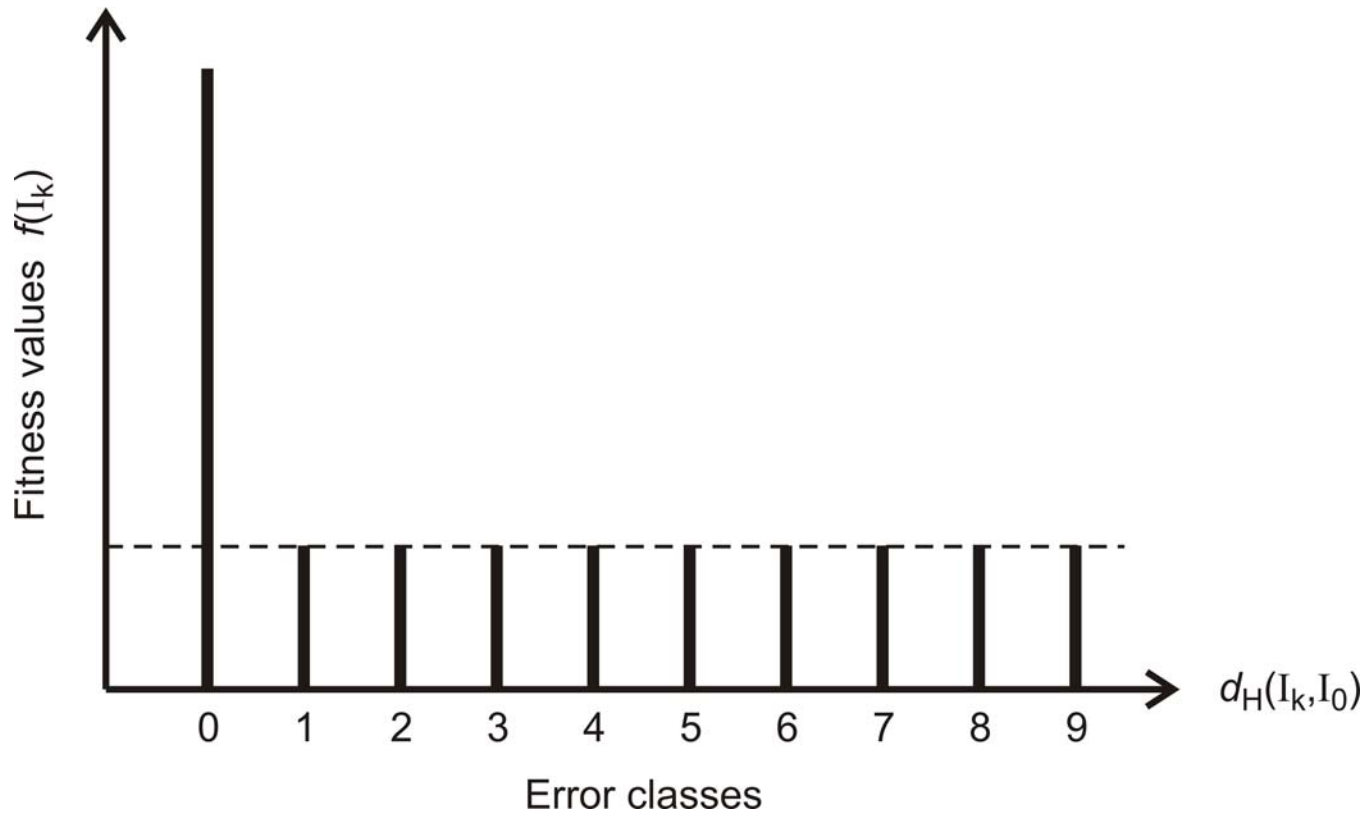
1. Origins of neutrality
2. RNA replication and quasispecies
3. **Selection on realistic landscapes**
4. Consequences of neutrality
5. Evolutionary optimization *in silico*



Complexity in molecular evolution



A fitness landscape showing an error threshold:

The single-peak landscape

Uniform error rate model:

$$Q_{ij} \;=\; p^{d_H(\mathbf{X}_i,\mathbf{X}_j)}\,(1-p)^{\left(n-d_H(\mathbf{X}_i,\mathbf{X}_j)\right)}$$

$d_H(\mathbf{X}_i, \mathbf{X}_j)$ ... Hamming distance

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

SELF-REPLICATION WITH ERRORS

A MODEL FOR POLYNUCLEOTIDE REPLICATION **

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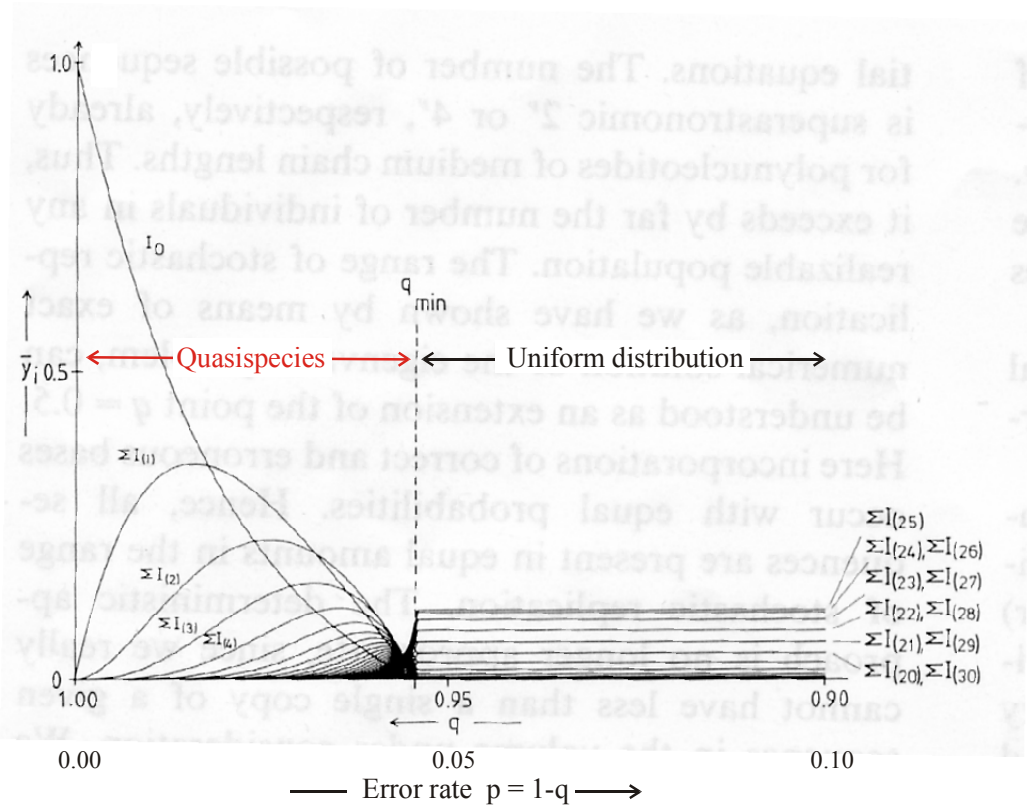
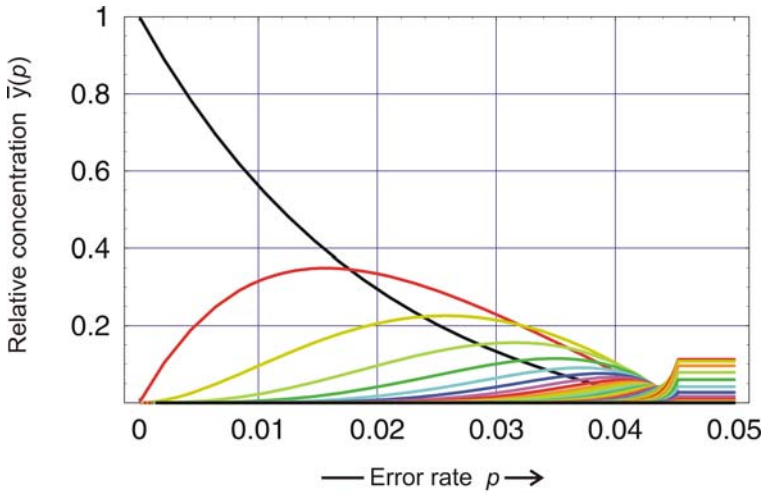
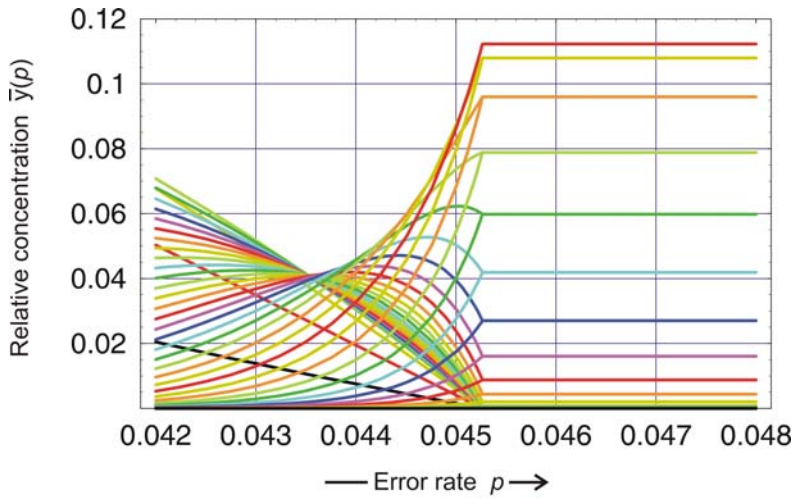
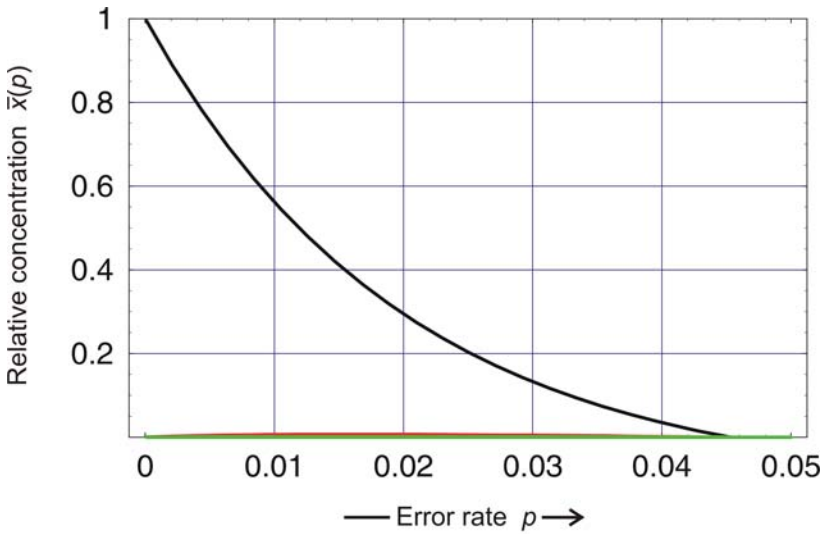
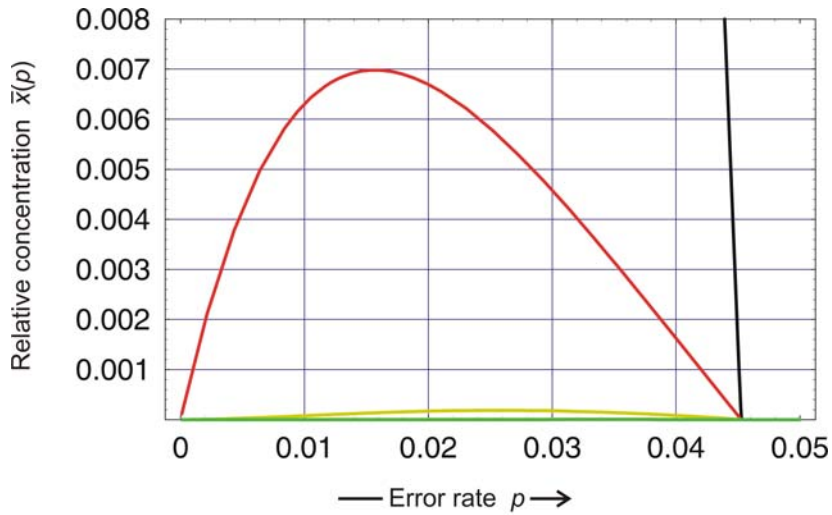


Stationary population or quasispecies as a function of the mutation or error rate *p*









Error threshold on a single peak fitness landscape with n = 50 and $\sigma = 10$

PHYSICAL REVIEW A VOLUME 45, NUMBER 8 15 APRIL 1992

Error thresholds for molecular quasispecies as phase transitions: From simple landscapes to spin-glass models

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(Received 19 June 1991)

The correspondence between Eigen's model [Naturwissenschaften **58**, 465 (1971)] for molecular quasispecies and the equilibrium properties of a lattice system proposed by Leuthäusser [J. Chem. Phys. **84**, 1884 (1986); J. Stat. Phys. **48**, 343 (1987)] is used to characterize the error thresholds for the existence of quasispecies as phase transitions. For simple replication landscapes the error threshold is related to a first-order phase transition smoothed by the complete wetting of the time surface. Replication landscapes based on the Hopfield Hamiltonian for neural networks allow for the tuning of the landscape complexity and reveal the existence of two error thresholds, bracketing a region of spin-glass quasispecies between the simple quasispecies and the fully disordered mixture of sequences.

PACS number(s): 87.10.+e, 64.60.Cn, 05.50.+q

VOLUME 91, NUMBER 13 PHYSICAL REVIEW LETTERS week ending 26 SEPTEMBER 2003

Equilibrium Distribution of Mutators in the Single Fitness Peak Model

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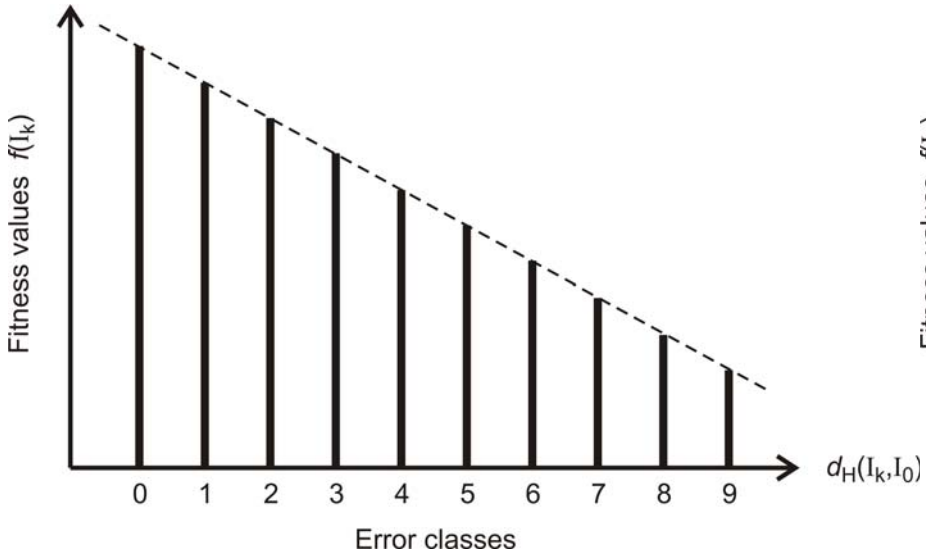
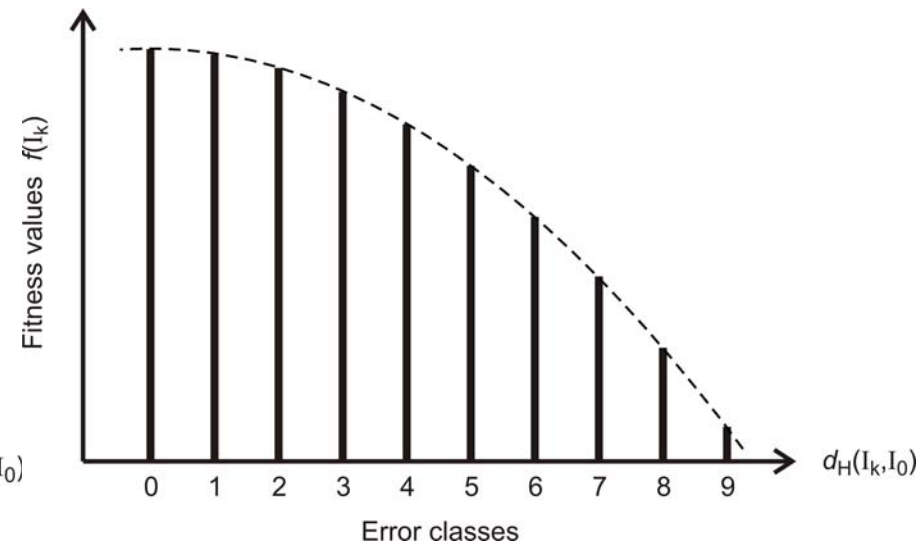
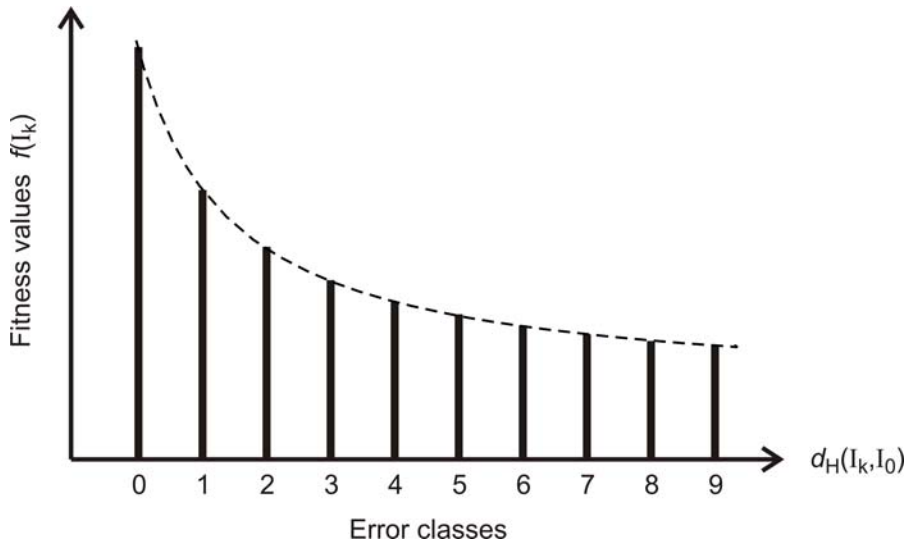
(Received 25 April 2003; published 26 September 2003)

This Letter develops an analytically tractable model for determining the equilibrium distribution of mismatch repair deficient strains in unicellular populations. The approach is based on the single fitness peak model, which has been used in Eigen's quasispecies equations in order to understand various aspects of evolutionary dynamics. As with the quasispecies model, our model for mutator-nonmutator equilibrium undergoes a phase transition in the limit of infinite sequence length. This "repair catastrophe" occurs at a critical repair error probability of $\epsilon_r = L_{\text{via}}/L$, where L_{via} denotes the length of the genome controlling viability, while L denotes the overall length of the genome. The repair catastrophe therefore occurs when the repair error probability exceeds the fraction of deleterious mutations. Our model also gives a quantitative estimate for the equilibrium fraction of mutators in *Escherichia coli*.

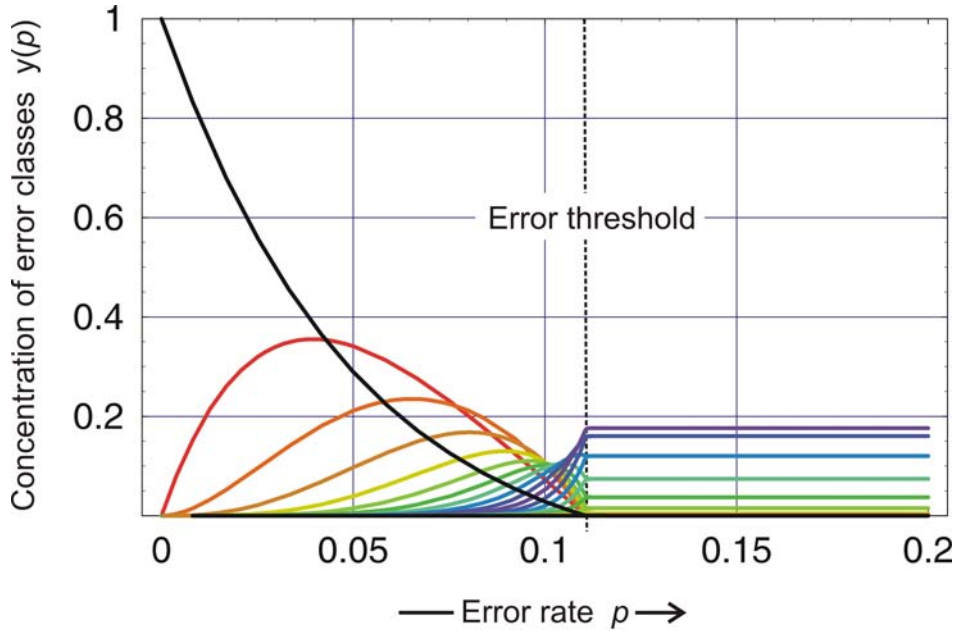
DOI: 10.1103/PhysRevLett.91.138105 PACS numbers: 87.23.Kg, 64.60.–i, 87.16.Ac

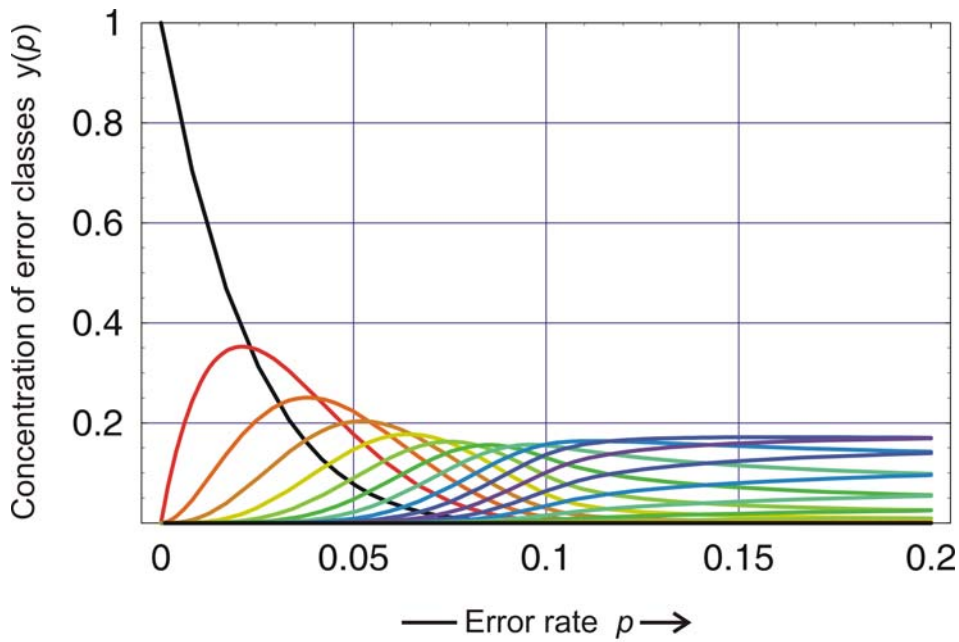






Fitness landscapes **not** showing error thresholds





Error thresholds and gradual transitions

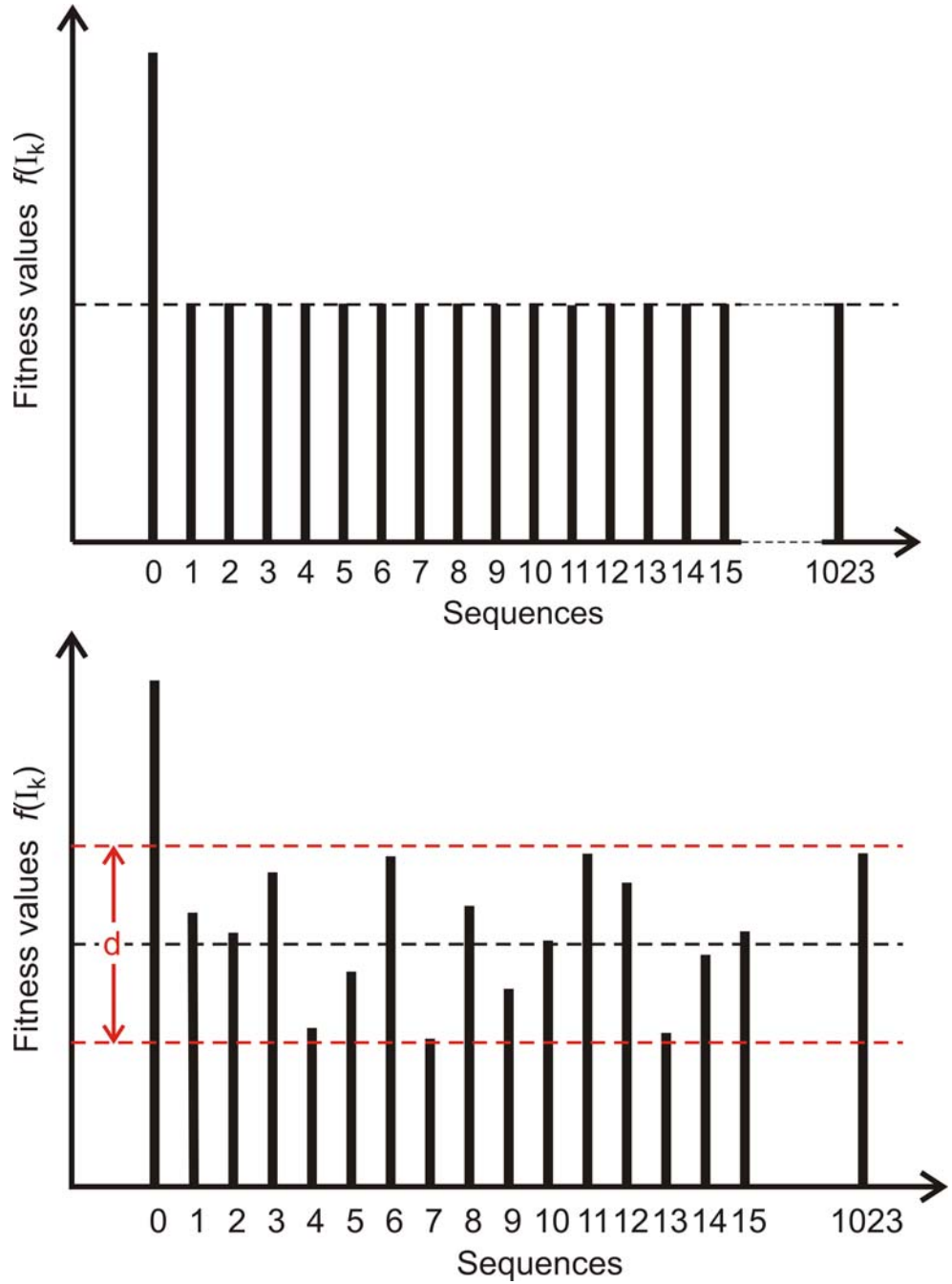
n = 20 and $\sigma = 10$

Features of realistic landscapes:

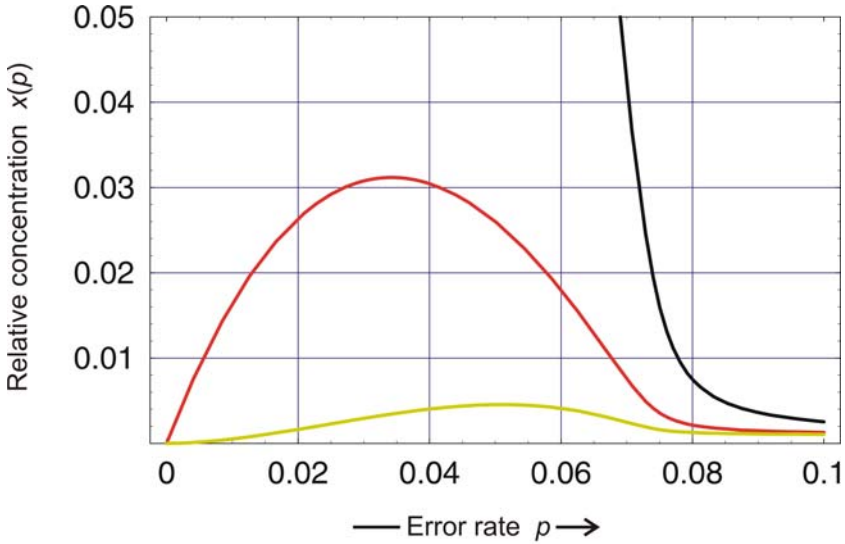
1. Variation in fitness values
2. Deviations from uniform error rates
3. Neutrality

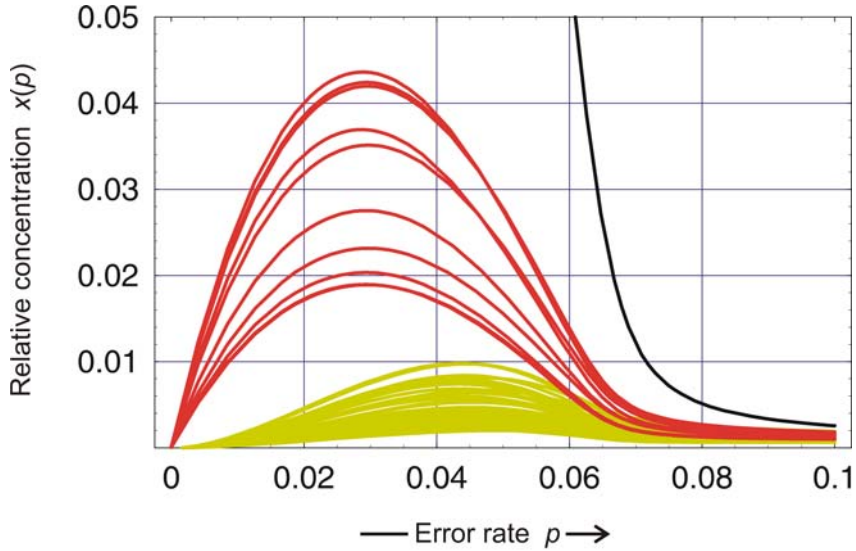
Features of realistic landscapes:

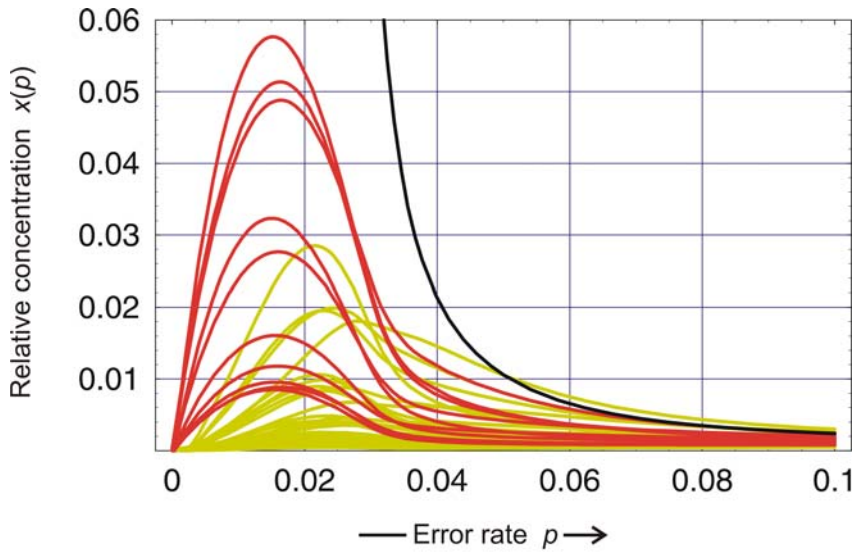
1. **Variation in fitness values**
2. Deviations from uniform error rates
3. Neutrality



Fitness landscapes showing error thresholds





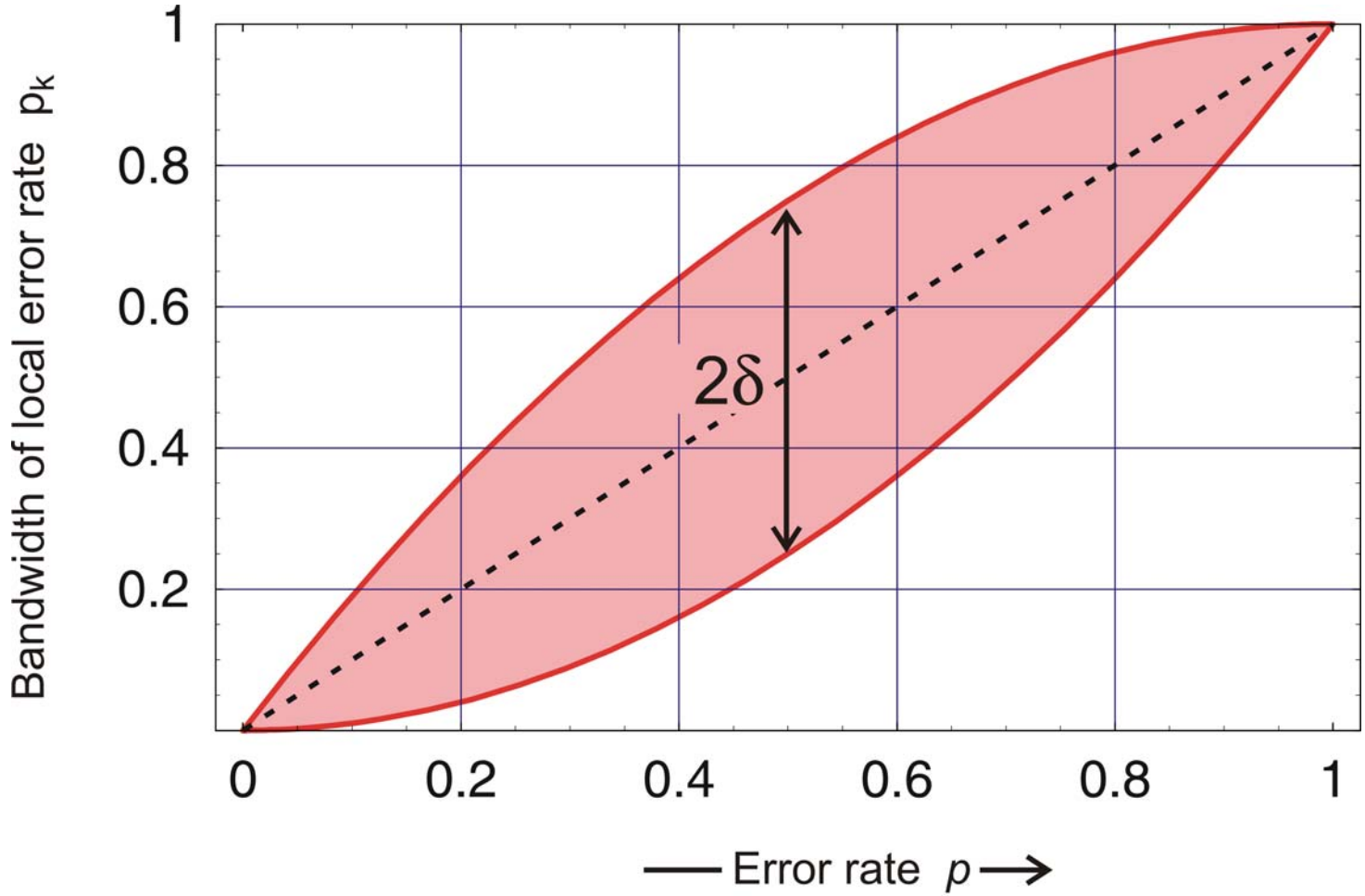


Error threshold: Individual sequences

n = 10, $\sigma = 2$ and d = 0, 1.0, 1.85

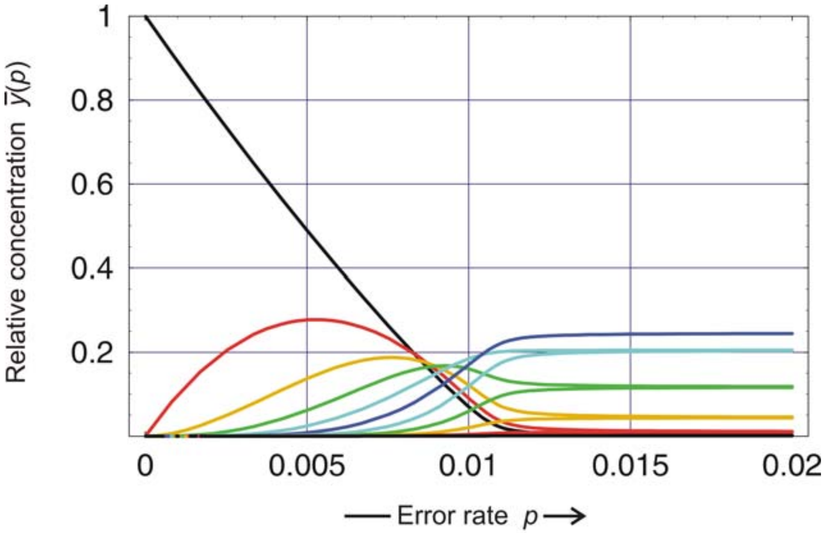
Features of realistic landscapes:

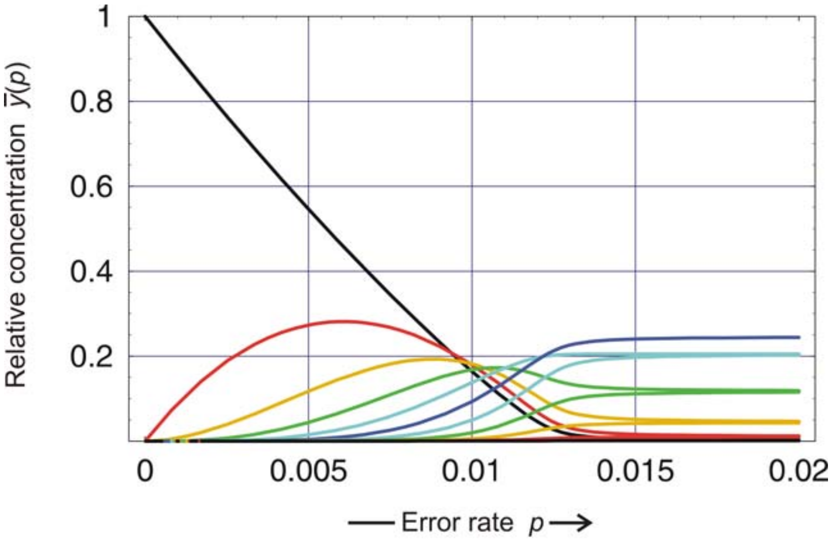
1. Variation in fitness values
2. **Deviations from uniform error rates**
3. Neutrality

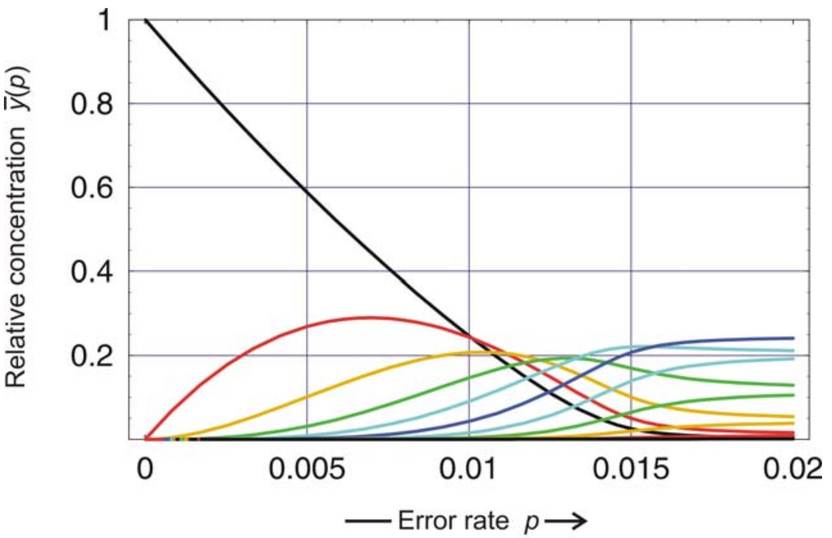


Local replication accuracy p_k:

$$p_k = p + 4\,\delta\,p(1-p)\,(X_{rnd}-0.5)\,,\quad k = 1,2,\ldots,2^{\nu}$$



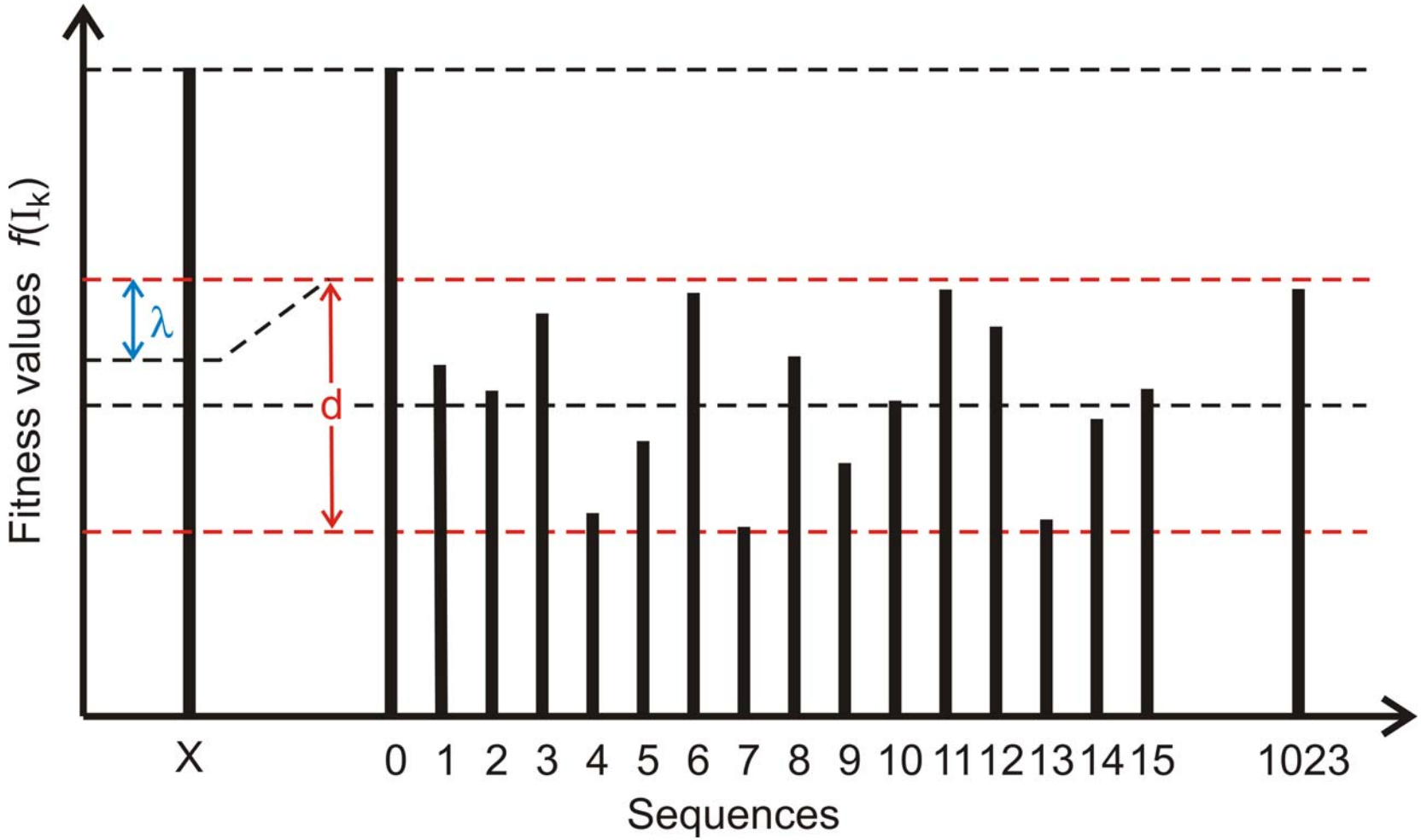




Error threshold: Classes

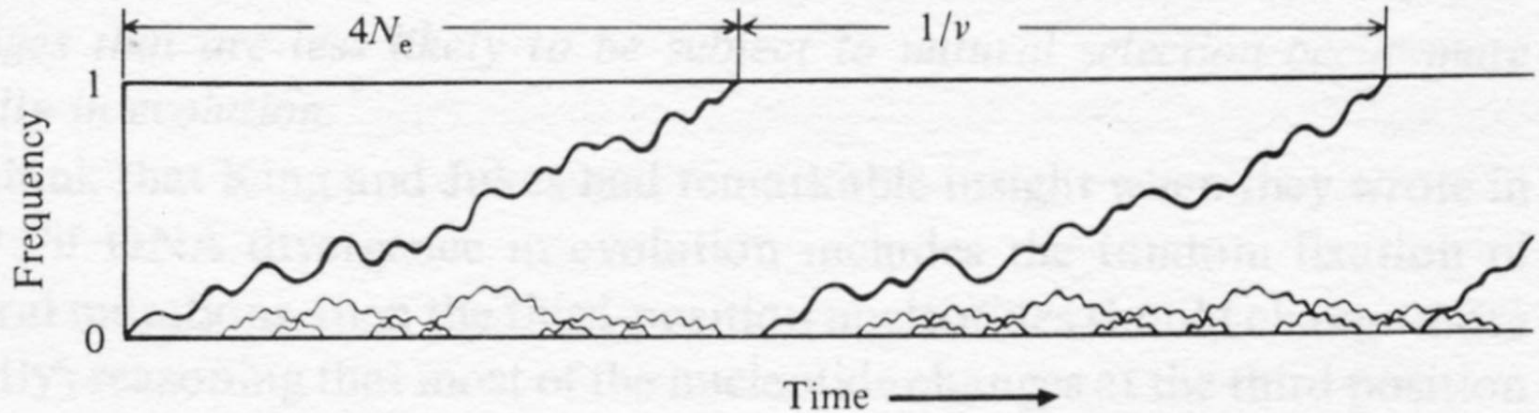
n = 10, σ = 1.1, δ = 0, 0.3, 0.5, and seed = 877

1. Origins of neutrality
2. RNA replication and quasispecies
3. Selection on realistic landscapes
4. **Consequences of neutrality**
5. Evolutionary optimization *in silico*



A fitness landscape including neutrality

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?

Bulletin of Mathematical Biology Vol. 50, No. 6, pp. 635–660, 1988.
Printed in Great Britain.

0092–8240/88$3.00 + 0.00
Pergamon Press plc
Society for Mathematical Biology

STATIONARY MUTANT DISTRIBUTIONS AND EVOLUTIONARY OPTIMIZATION

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Währingerstraße 17,
A 1090 Wien,
Austria

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.

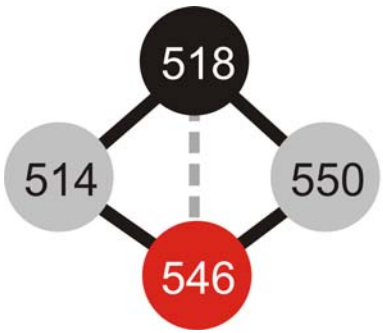
In addition we investigate the time dependence of the mean excess production as a function of initial conditions. Although it is optimized under most conditions, cases can be found which are characterized by decrease or non-monotonous change in mean excess productions.

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

$\lambda = 0.01,\ s = 877$

$\mathbf{d_H = 2}$

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

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

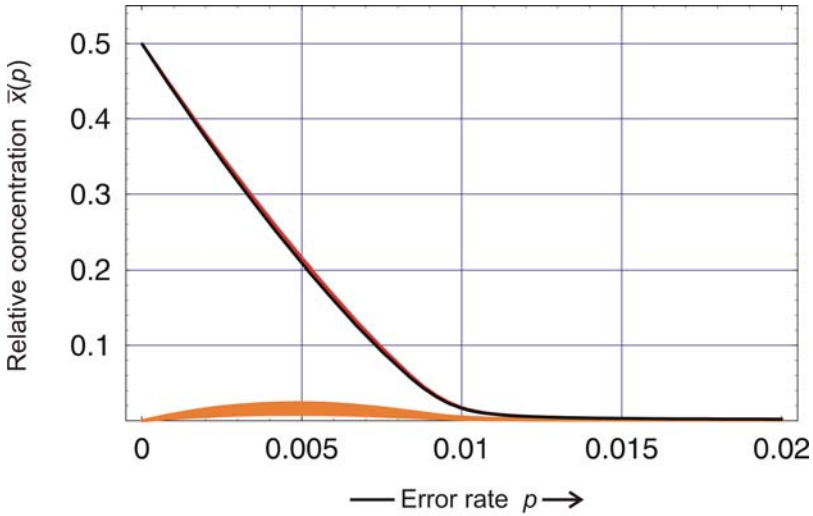
$\mathbf{d_H \geq 3}$

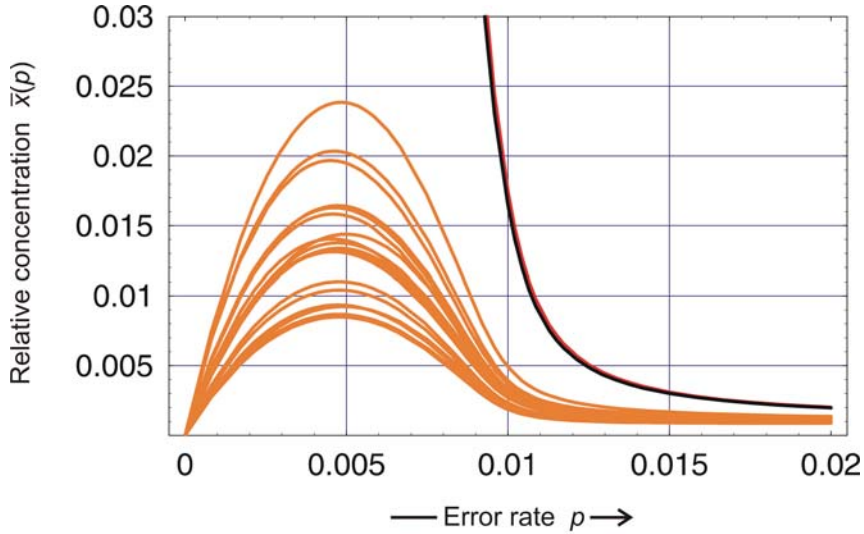
$$\lim_{p\to 0} x_1(p) = 1, \lim_{p\to 0} x_2(p) = 0 \quad \text{or}$$

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

Pairs of genotypes in neutral replication networks

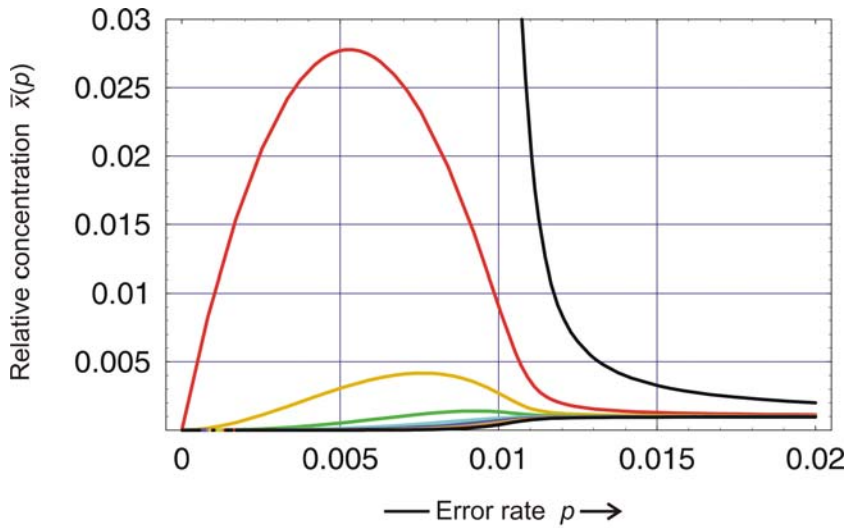
Random fixation in the sense of Motoo Kimura





Neutral network

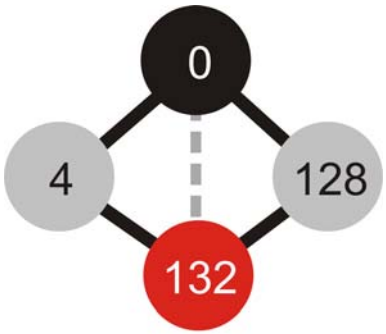
$\lambda = 0.01,\ s = 367$



Neutral network: Individual sequences

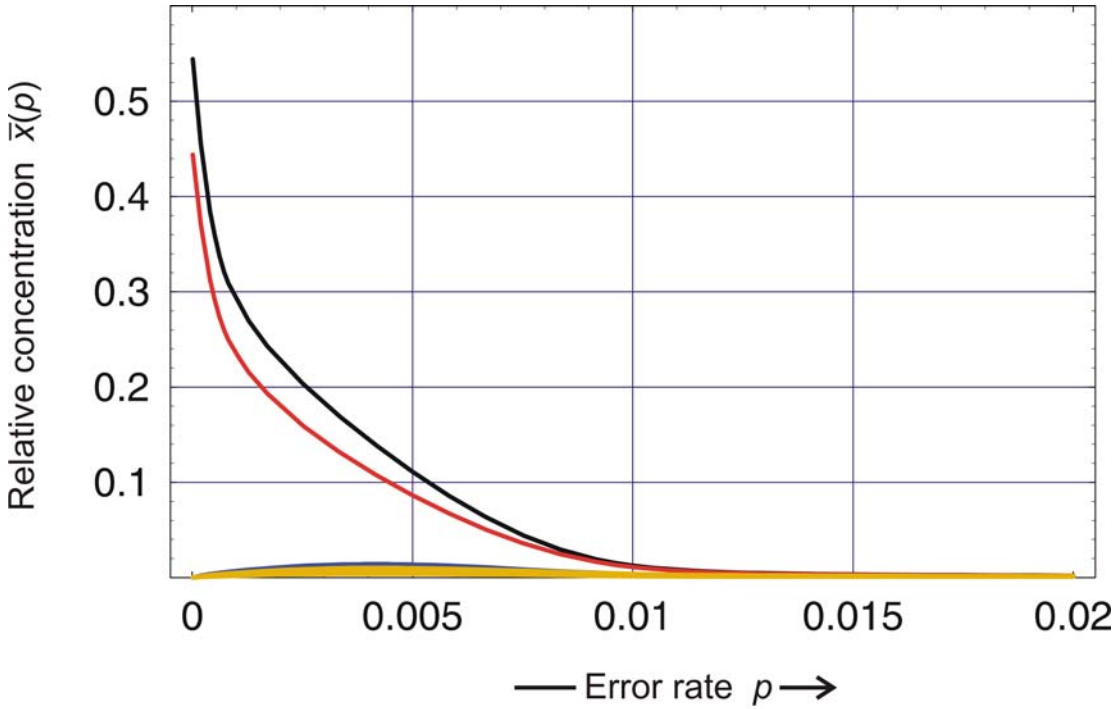
$n = 10,\ \sigma = 1.1,\ d = 1.0$

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



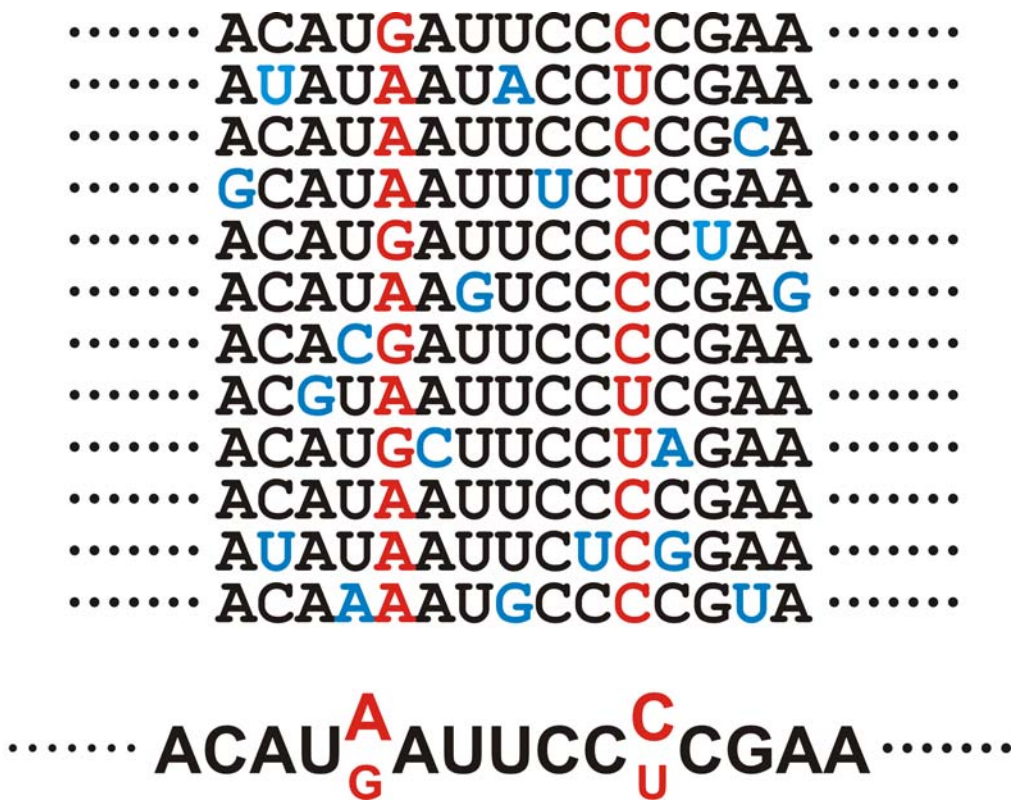
Neutral network

$\lambda = 0.01,\ s = 877$

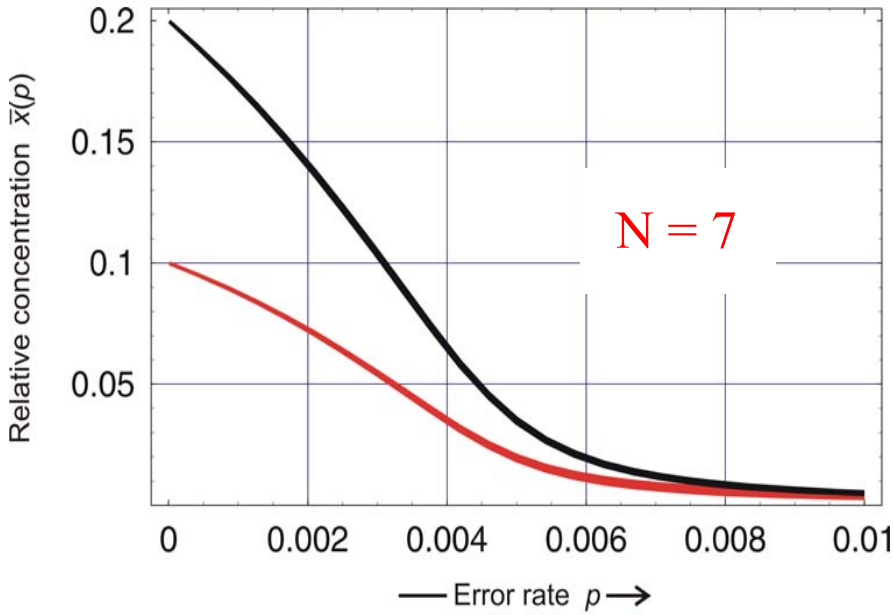


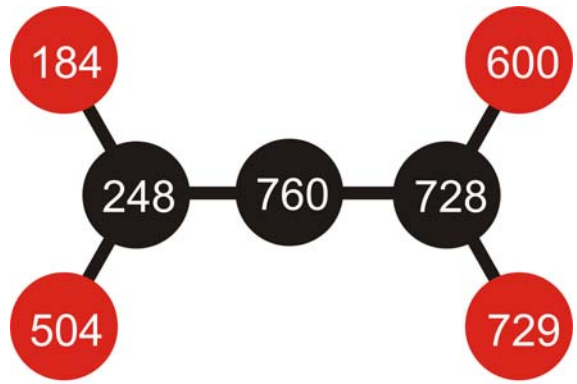
Neutral network: Individual sequences

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



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

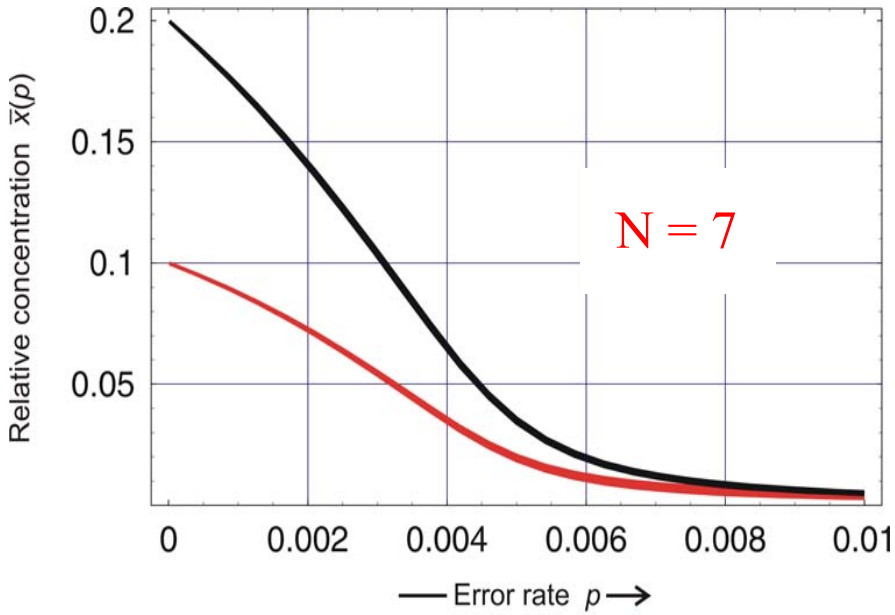


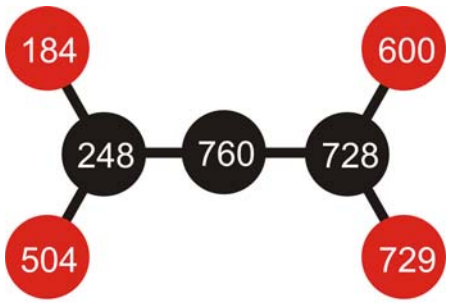


Neutral network

$\lambda = 0.10$, s = 229

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





Neutral network

λ = 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

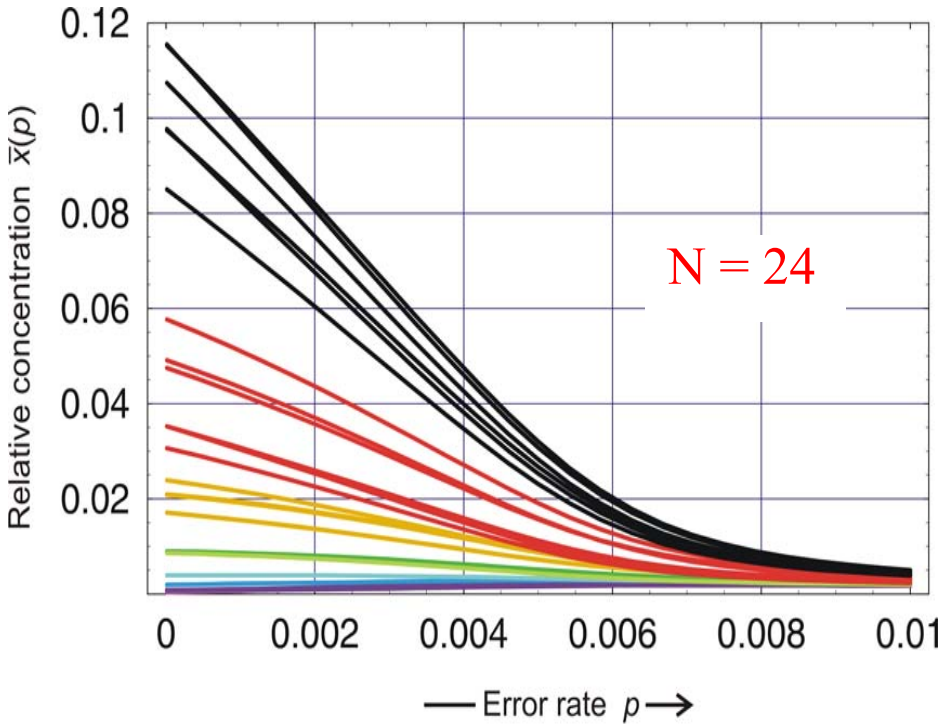
$$\lambda_0 = f + 2\varepsilon ,$$

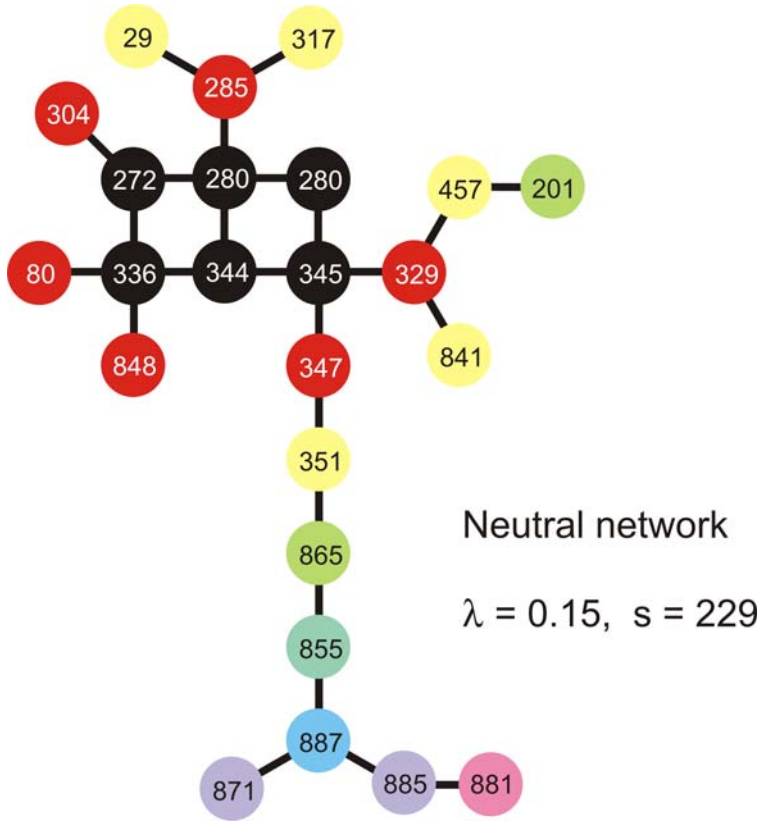
$$\lambda_1 = f + \sqrt{2}\varepsilon ,$$
$$\lambda_{2,3,4} = f ,$$
$$\lambda_5 = f - \sqrt{2}\varepsilon ,$$
$$\lambda_6 = f - 2\varepsilon .$$

Largest eigenvector of W

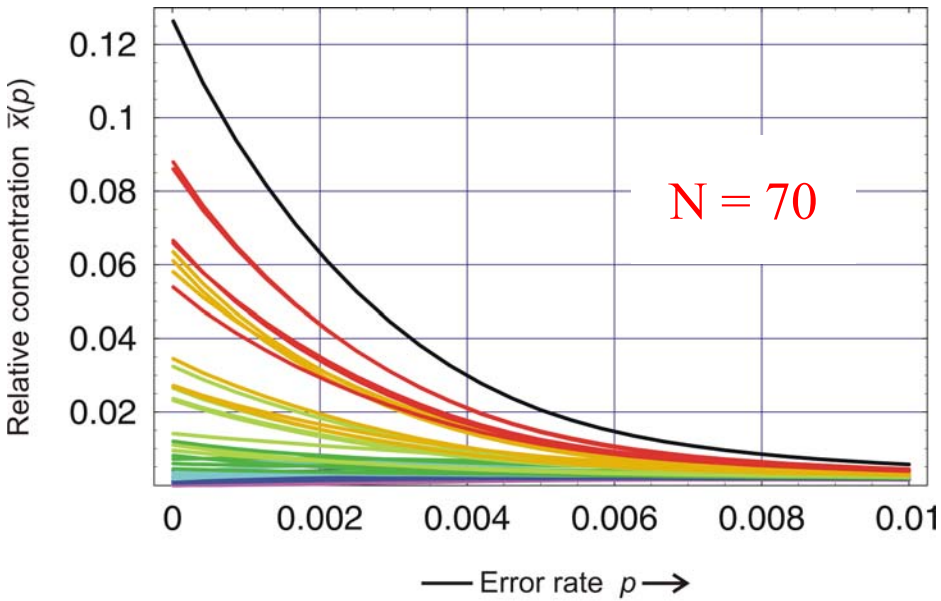
$$\xi_0 = (0.1, 0.1, 0.2, 0.2, 0.2, 0.1, 0.1) .$$

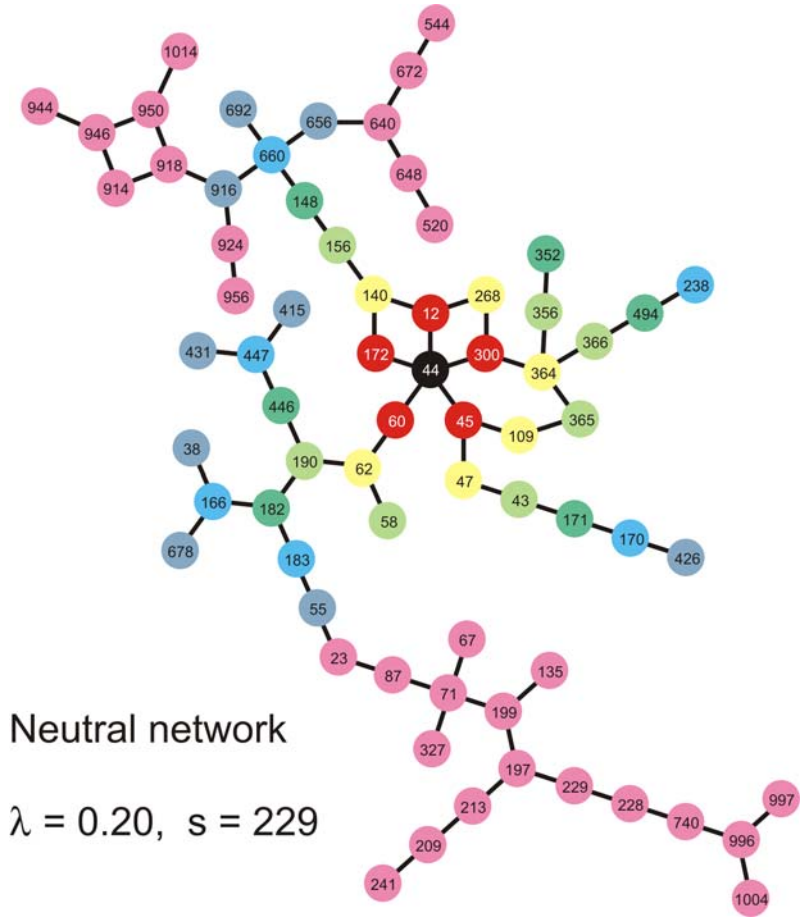
Neutral networks with increasing λ: λ = 0.10, s = 229





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





Neutral network

$\lambda = 0.20,\ s = 229$

Neutral networks with increasing λ: $\lambda = 0.20$, $s = 229$

1. Origins of neutrality
2. RNA replication and quasispecies
3. Selection on realistic landscapes
4. Consequences of neutrality
5. **Evolutionary optimization *in silico***

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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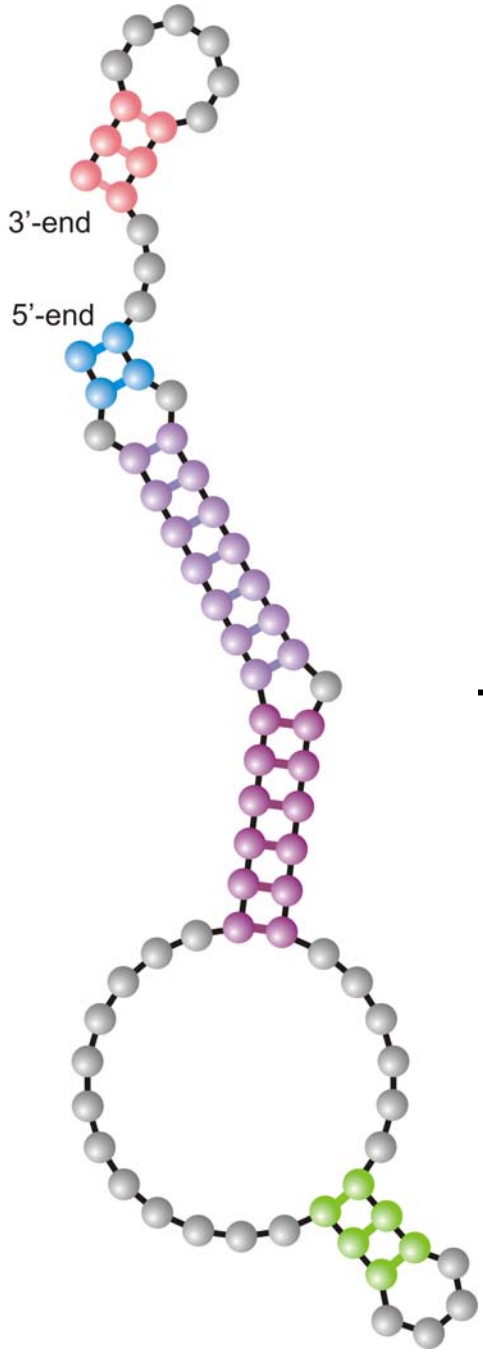
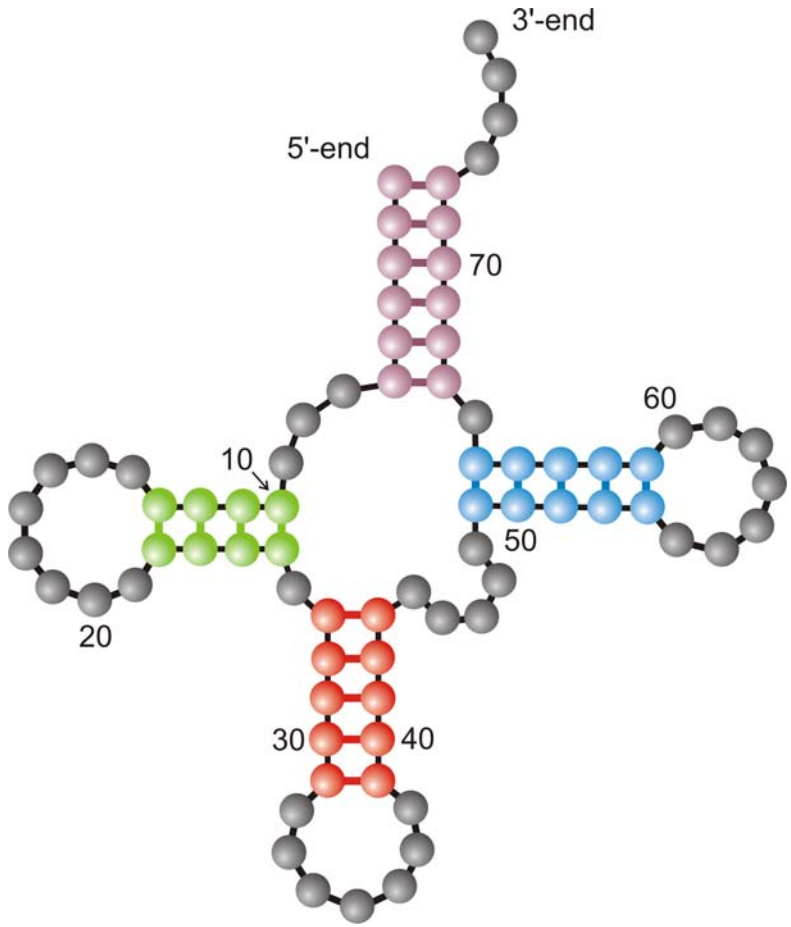
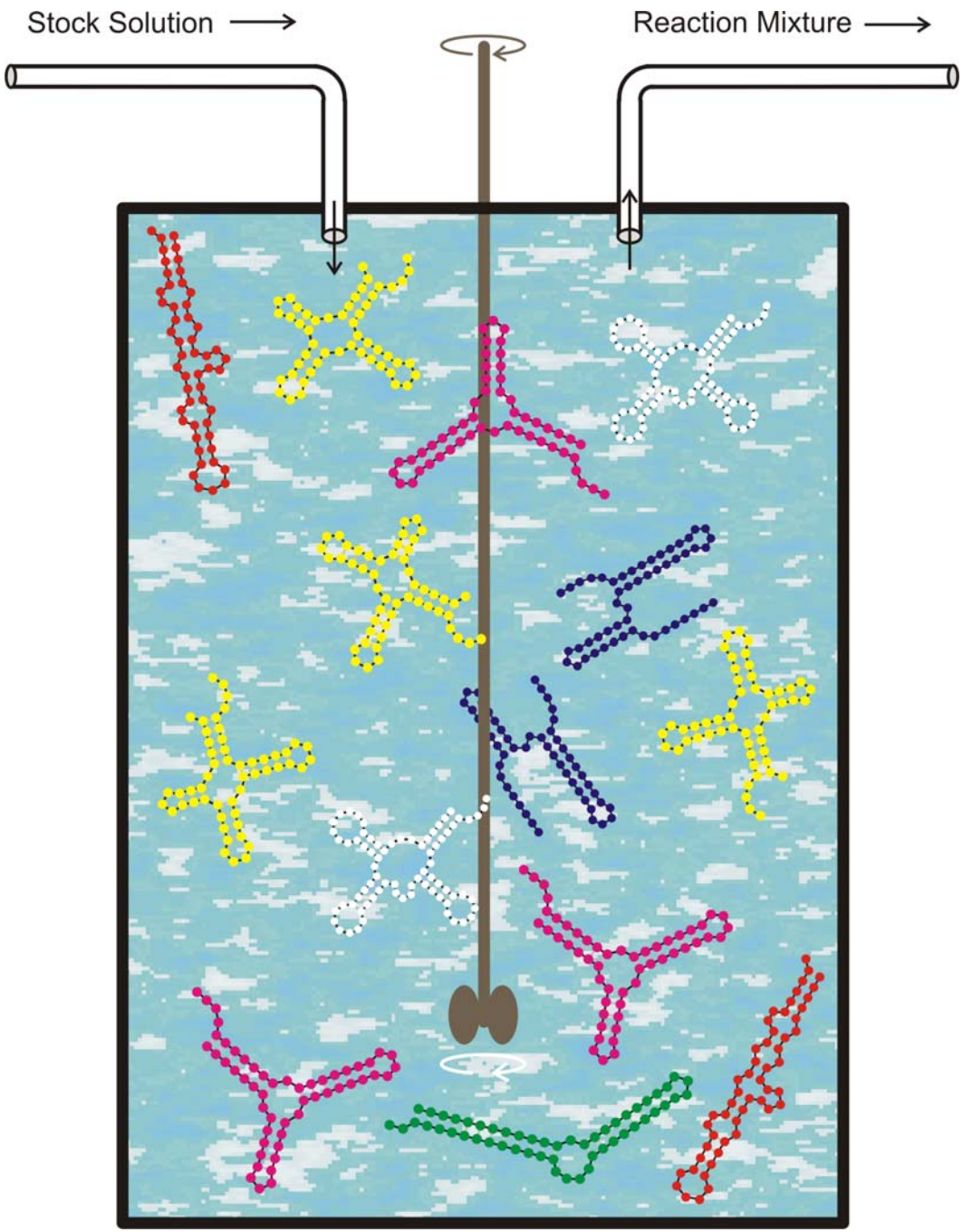


Structure of randomly chosen initial sequence



Phenylalanyl-tRNA as target structure



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,

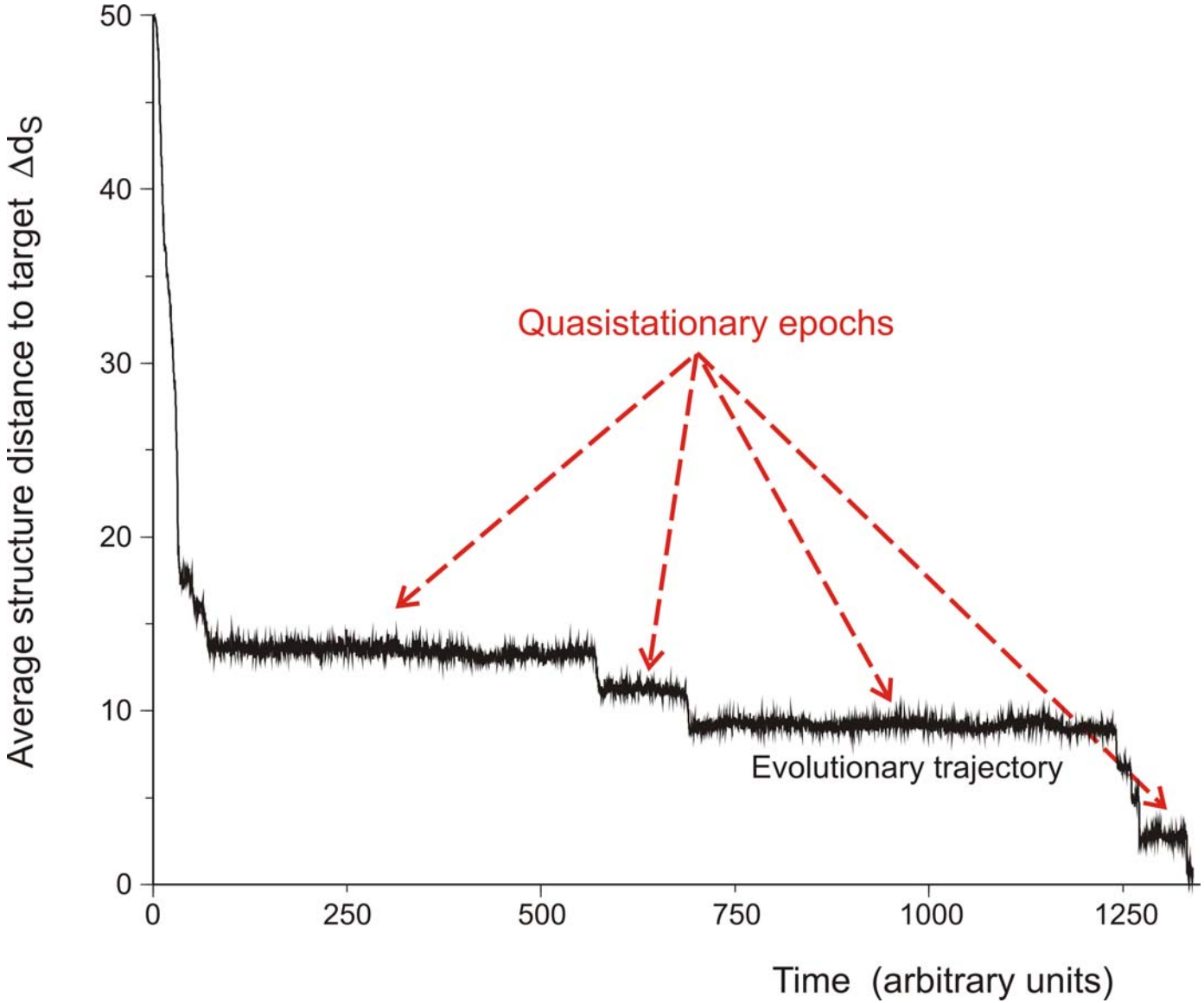
is determined by the flux:

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

Mutation rate:

p = 0.001 / Nucleotide × Replication

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

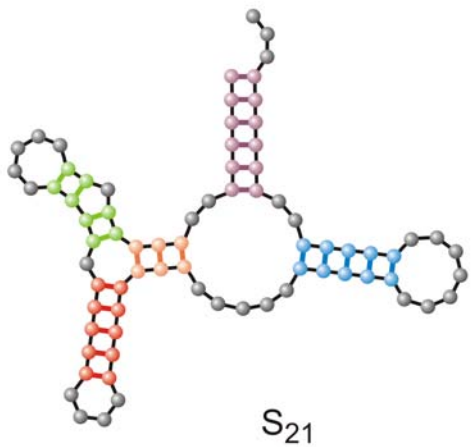


In silico optimization in the flow reactor: Evolutionary Trajectory

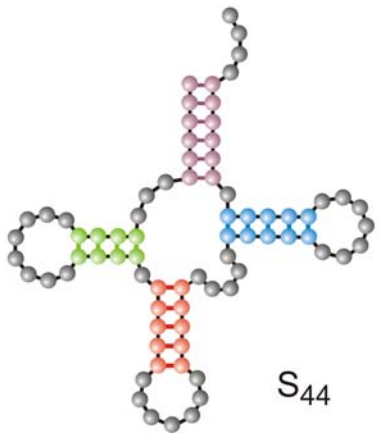
Randomly chosen
initial structure

S_0

S_9



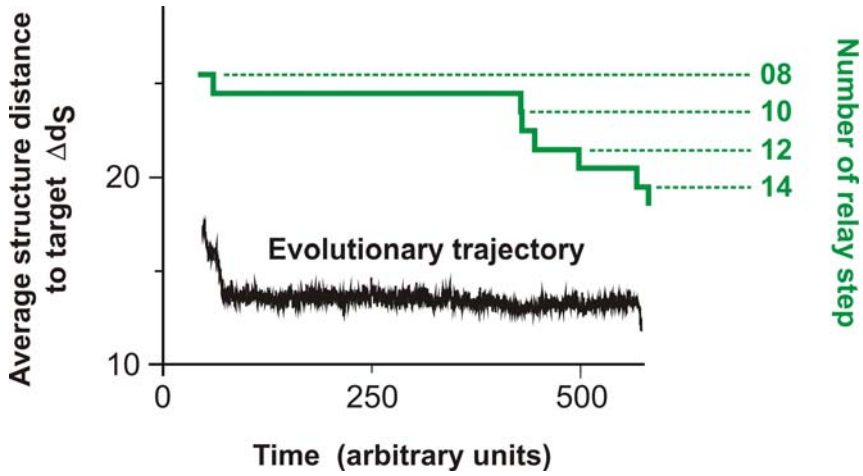
S_{21}



S_{44}

Phenylalanyl-tRNA
as target structure

28 neutral point mutations during a long quasi-stationary epoch

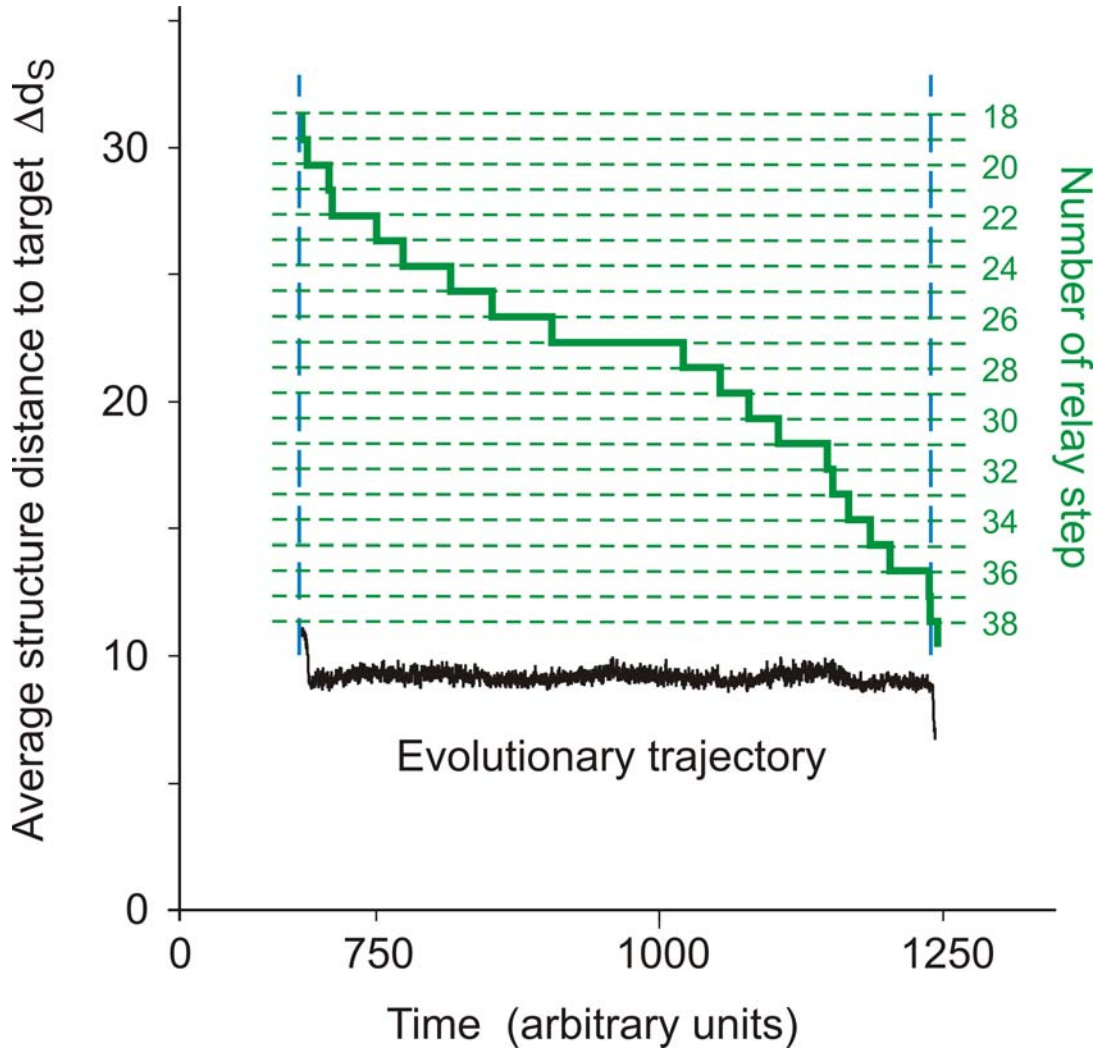


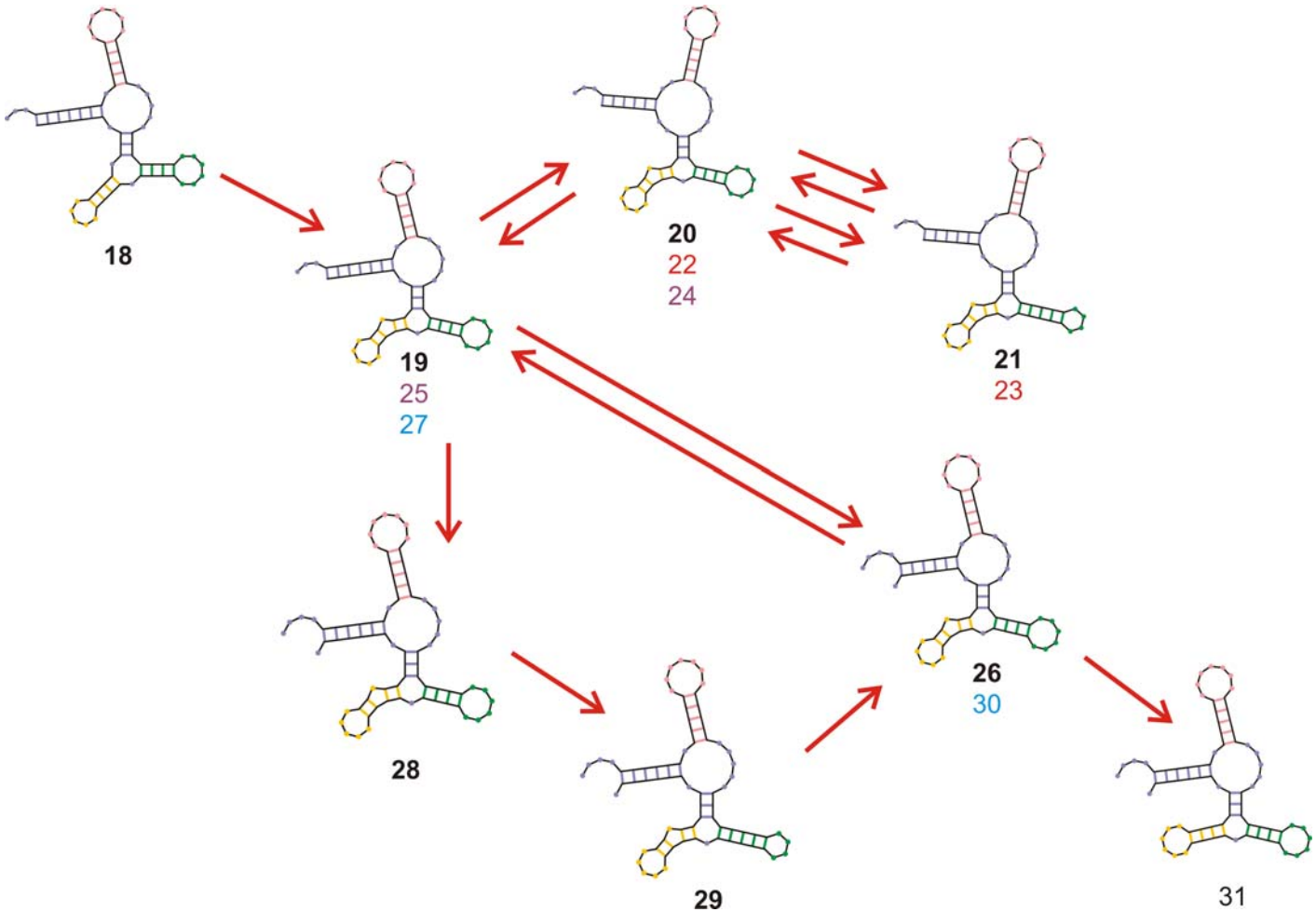

```
entry   GGUAUGGGCGUUGAAUAGUAGGGUUUAAACCAAUCGGCCAACGAUCUCGUGUGCGCAUUUCAUAUCCCGUACAGAA
8       .((((((((((((.........(((....)))).......)))))).....((((.......))))))))))....
exit    GGUAUGGGCGUUGAAUAAUAGGGUUUAAACCAAUCGGCCAACGAUCUCGUGUGCGCAUUUCAUAUCCCAUACAGAA
entry   GGUAUGGGCGUUGAAUAAUAGGGUUUAAACCAAUCGGCCAACGAUCUCGUGUGCGCAUUUCAUAUACCAUACAGAA
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

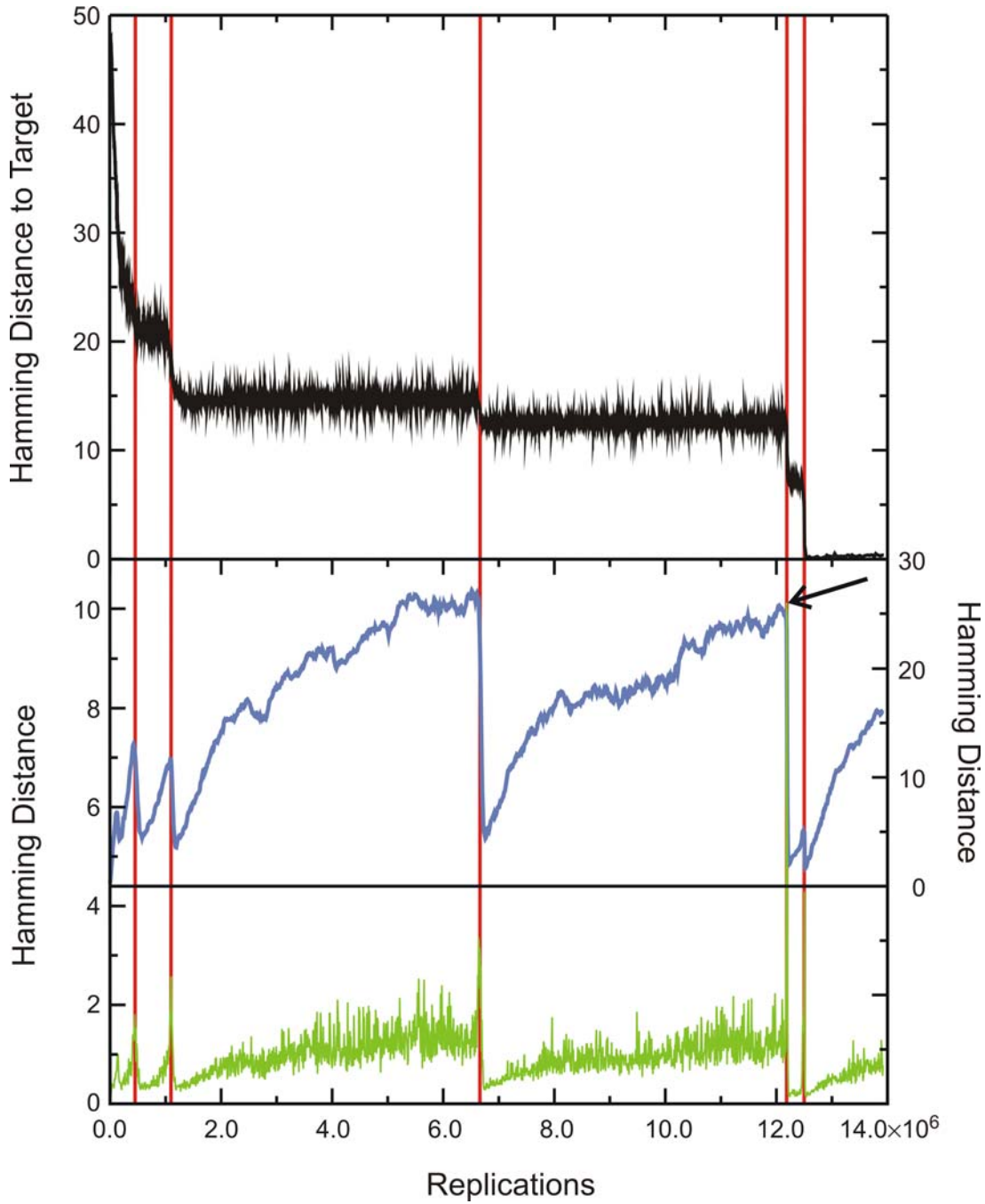

Average structure distance to target Δd_S
30
20
10
0
0
750
1000
1250
Time (arbitrary units)
Evolutionary trajectory
18
20
22
24
26
28
30
32
34
36
38
Number of relay step


18
19
25
27
20
22
24
21
23
28
29
26
30
31

Evolutionary trajectory

Spreading of the population on neutral networks

Drift of the population center in sequence space



Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 397–401, January 1996
Evolution

Smoothness within ruggedness: The role of neutrality in adaptation

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Communicated by Hans Frauenfelder, Los Alamos National Laboratory, Los Alamos, NM, September 20, 1995 (received for review June 29, 1995)

ABSTRACT **RNA secondary structure folding algorithms predict the existence of connected networks of RNA sequences with identical structure. On such networks, evolving populations split into subpopulations, which diffuse independently in sequence space. This demands a distinction between two mutation thresholds: one at which genotypic information is lost and one at which phenotypic information is lost. In between, diffusion enables the search of vast areas in genotype space while still preserving the dominant phenotype. By this dynamic the success of phenotypic adaptation becomes much less sensitive to the initial conditions in genotype space.**

To explain the high fixation rate of nucleotide substitutions in a population, Kimura (1) argued that the vast majority of genetic change at the level of a population must be neutral rather than adaptive. Sewall Wright's reaction to Kimura's point was politely neutral (ref. 2, p. 474): "Changes in wholly nonfunctional parts of the molecule would be the most frequent ones but would be unimportant, unless they occasionally give a basis for later changes which improve function in the species in question which would then become established by selection." Today, in view of the data generated by comparative sequence analysis, the surprise is no longer over the existence of neutrality but over how little conservation there is at the sequence level (3–6). This makes Wright's point even more pertinent. How are we to imagine the relation between neutral evolution and adaptation? An answer to this question requires a model of the relationship between genotype and phenotype. Such a model is available for RNA secondary structure. The latter can be computed from the sequence by means of procedures based on thermodynamic data which have become standard in the past 15 years (7, 8). Secondary structure covers the major share of the free energy of tertiary structure formation and is frequently used to interpret RNA function and evolutionary data. As such, the case is a qualitatively important one.

Robust Properties of RNA Folding

The mapping from sequences to secondary structures is many to one for two reasons: (*i*) there are many more sequences than secondary structures, and (*ii*) some structures are realized much more frequently than others (9). Call two sequences connected if they differ by one or at most two point mutations. A neutral network, then, is a set of sequences with identical structure so that each sequence is connected to at least one other sequence. The crucial point for our discussion comes from a recent study of the standard secondary structure prediction algorithm (9), which showed that such networks exist and that for frequent structures these networks percolate through sequence space. For example, starting at a sequence that folds into a tRNA structure, it is possible to traverse sequence space along a connected path, thus changing every nucleotide position without ever changing the structure. Moreover, due to the high-dimensionality of sequence space, networks of frequent structures penetrate each other so that each frequent structure is almost always realized within a small distance of any random sequence. These features seem to be intrinsic to RNA folding, since they are insensitive to whether the folding algorithm is thermodynamic, kinetic, or maximum matching (E. Bornberg-Bauer, M. Tacker, and P. Schuster, personal communication) or whether one considers one minimum free energy structure or the entire Boltzmann ensemble (10).

A Simple Model for Test Tube Evolution

To assess the consequences of these properties for molecular evolution, we study a model in which the replication rate (fitness) of an RNA sequence depends on its secondary structure. Our folding procedure¶ is a speed-tuned implementation of the Zuker–Stiegler algorithm (8). The model consists of a population of RNA sequences of fixed length ν, which replicate and mutate in a stirred flow reactor. RNA populations manageable in the computer or in the laboratory are tiny compared to the size of the sequence space (4^ν), and a correct simulation must, therefore, resort to stochastic chemical reaction kinetics (11, 12). A selection pressure is induced by a dilution flow, which adjusts over time to keep the total RNA population fluctuating around a constant capacity N (11, 13). This setup mimics Spiegelman's serial transfer technique (14), where sequences with a replication rate above (below) the average increase (decrease) in concentration.

When a sequence undergoes a replication, each base is copied with fidelity $1 - p$. The overall replication rate of an individual sequence is defined to be a function of the distance (9, 30) between its secondary structure and a predefined target structure. Here the target structure is the $tRNA^{Phe}$ cloverleaf, but the structure of any randomly chosen sequence would do as well. This corresponds to the artificial *in vitro* selection of a structure with some desired function or affinity to a target (14–21). A similar situation, though with proteins and not RNA, occurs in the affinity maturation of the immune response (22). In both artificial and natural selection there are two sources of neutrality: one is the sequence (genotype) to structure (phenotype) mapping, and the other is the structure to replication rate (fitness) mapping. It is the former source that is central to this discussion. Notice, thus, that in the present model the second source of neutrality arises only for sequences whose structures differ from the target.

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§To whom reprint requests should be addressed at Institut für Theoretische Chemie, Währinger Strasse 17, A-1090 Vienna, Austria.

¶Hofacker, I. L., Fontana, W., Stadler, P. F., and Schuster, P. RNA folding package available by anonymous ftp from ftp.itc.univie.ac.at in/pub/RNA.

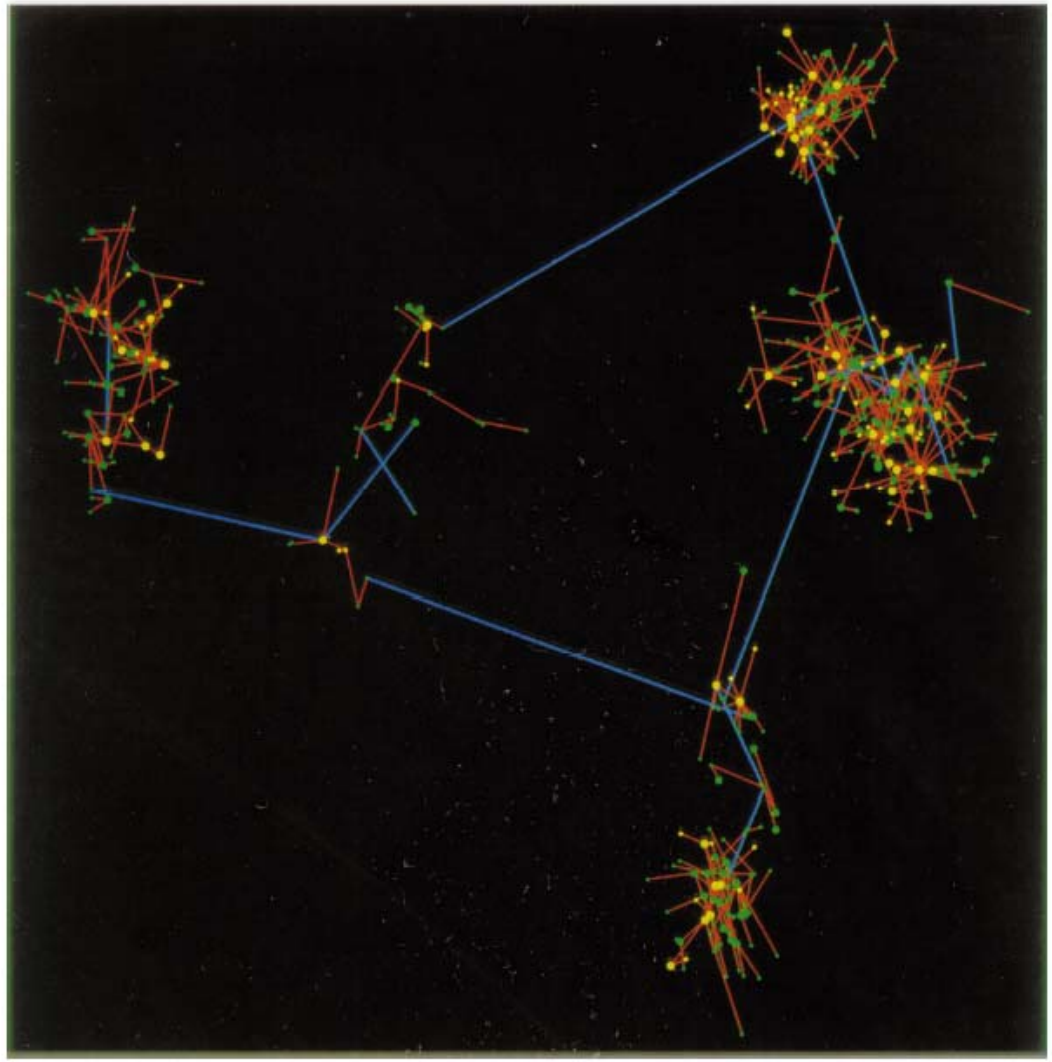
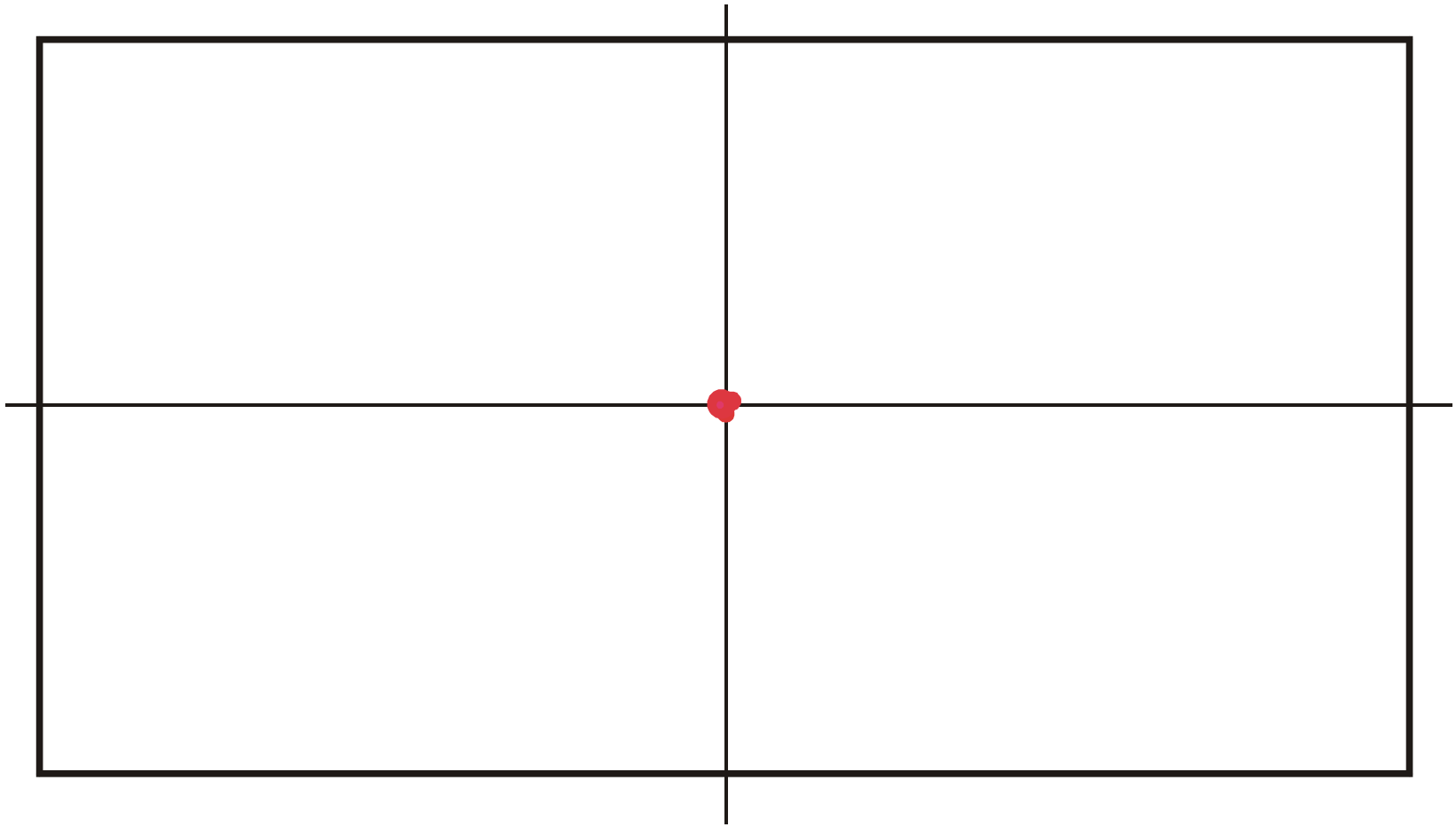
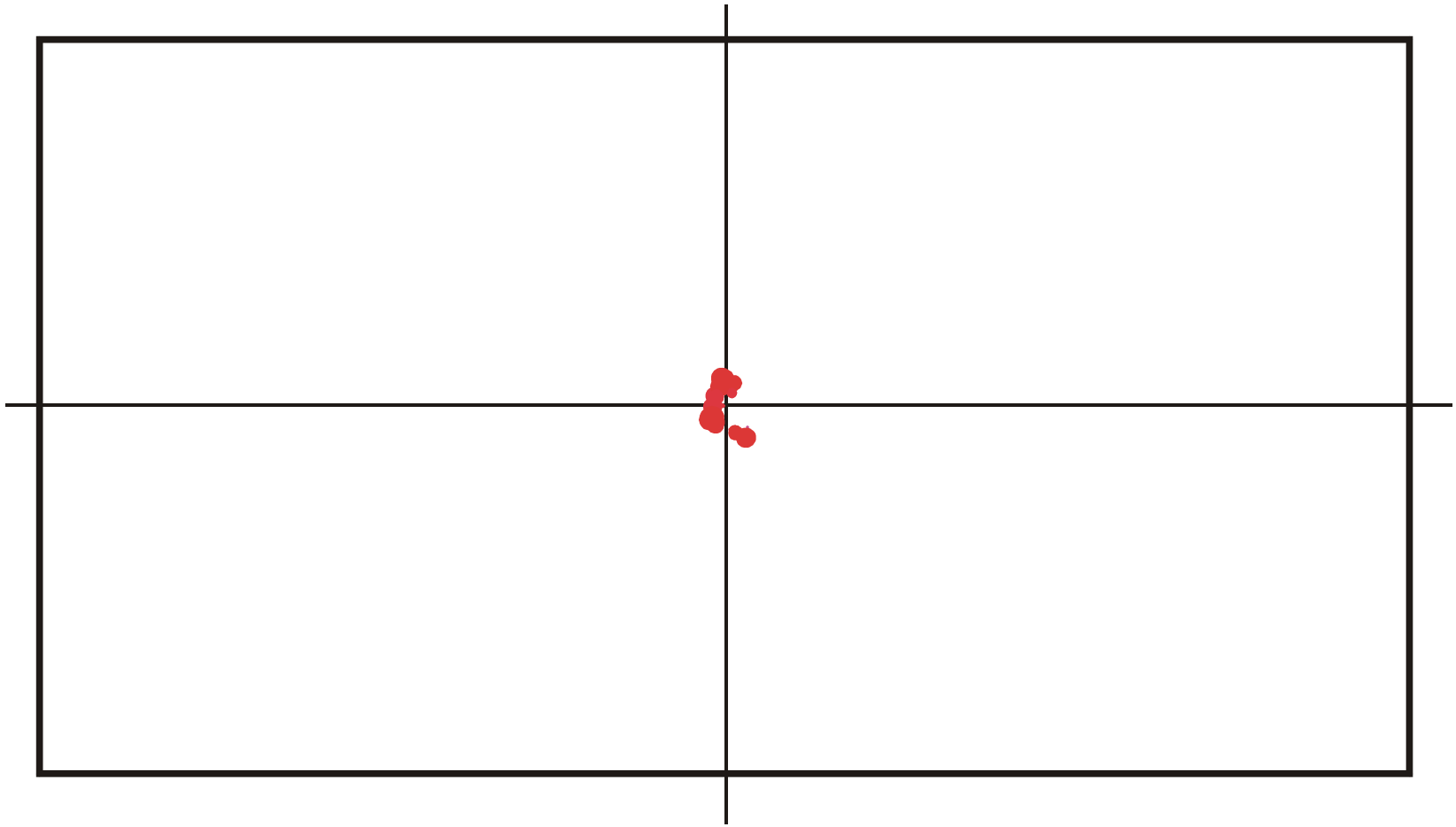


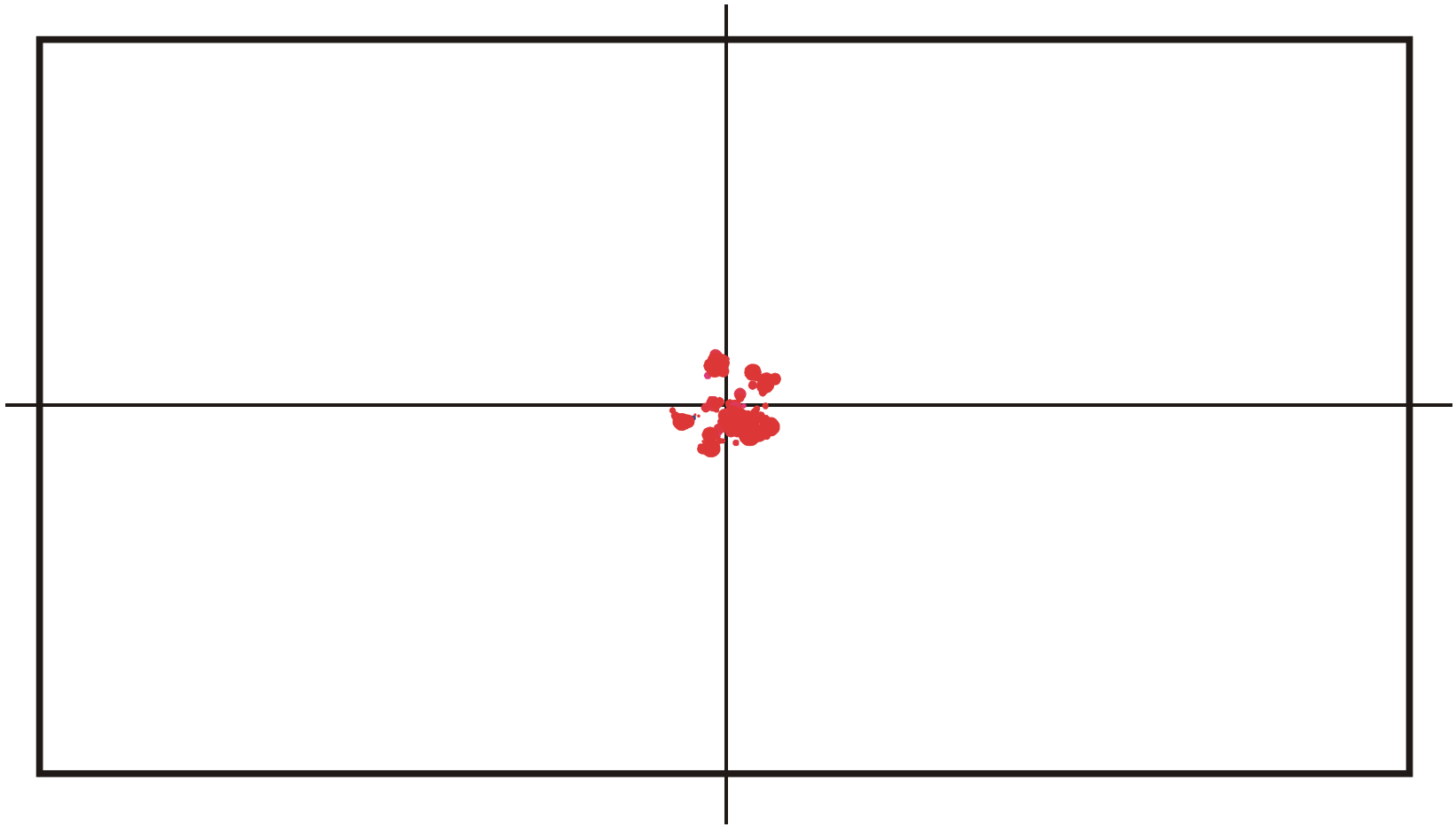
FIG. 2. Population structure in sequence space. The support of a population in sequence space is the set of sequences present in at least one copy. The population support can be pictured in two dimensions using some theorems from distance geometry (27). We compute the metric matrix M with entries $m_{ij} = (d_{0i}^2 + d_{0j}^2 - d_{ij}^2)/2$, where d_{ij} is the Hamming distance between sequences i and j and 0 is the center of mass of the support. Sequences are expressed in principal axes coordinates by diagonalizing M. Only the components corresponding to the largest two eigenvalues are kept, yielding a projection onto the plane that captures most of the variation. Dots represent a static snapshot of $N = 2000$ individuals after 135 time units replicating with $p = 0.002$. Among the 2000 individuals, 631 are different and among them 301 fold into different structures. To help correct for the distortions of the projection, the dots are connected by the edges of the minimum spanning tree. Edges connect closest points. Red (blue), Hamming distance less (more) than 6; dot size large (small), more (less) than four copies in the population; yellow (green), sequences that do (do not) fold into the tRNA target structure.



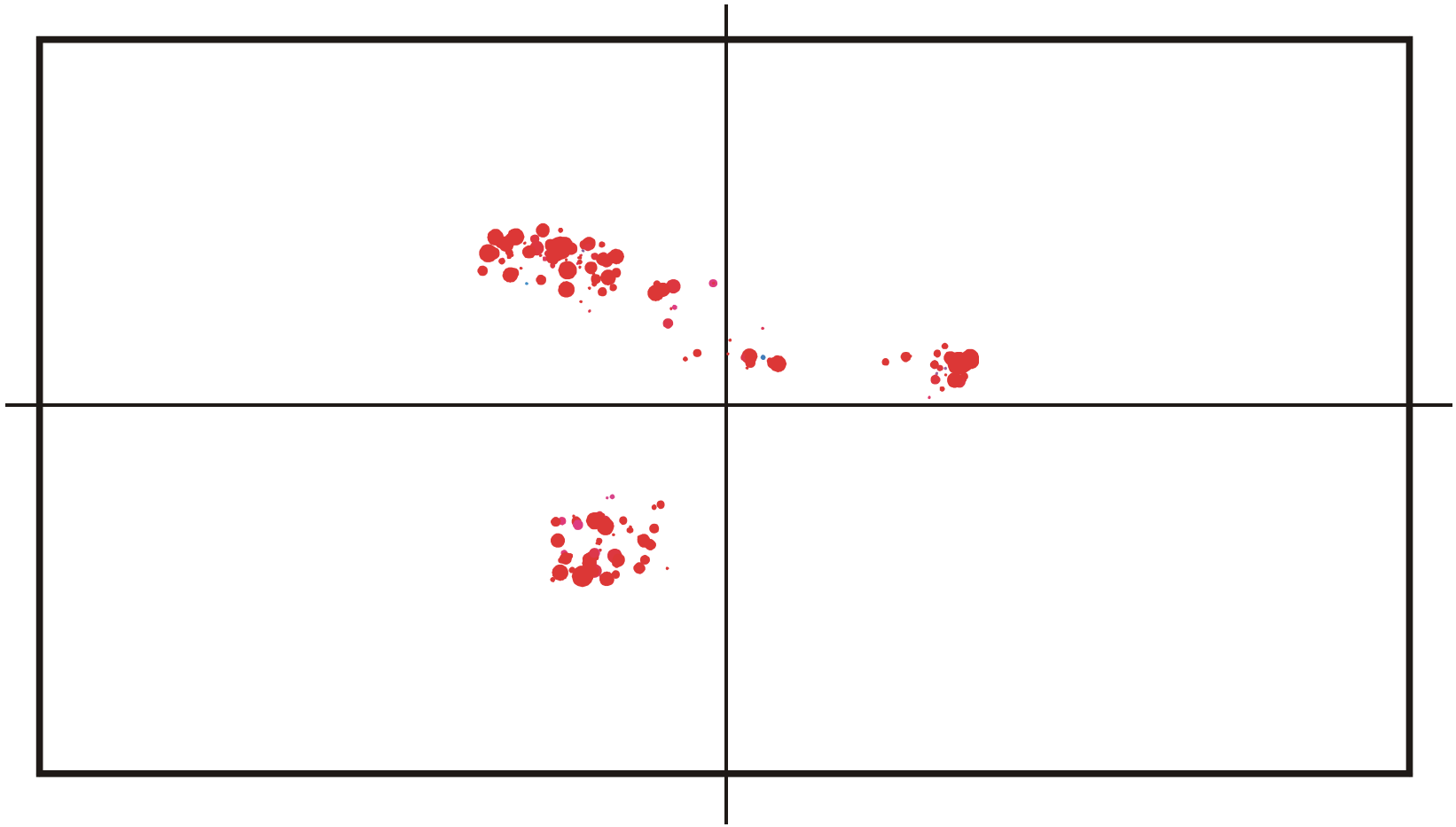
Spreading and evolution of a population on a neutral network: t = 150



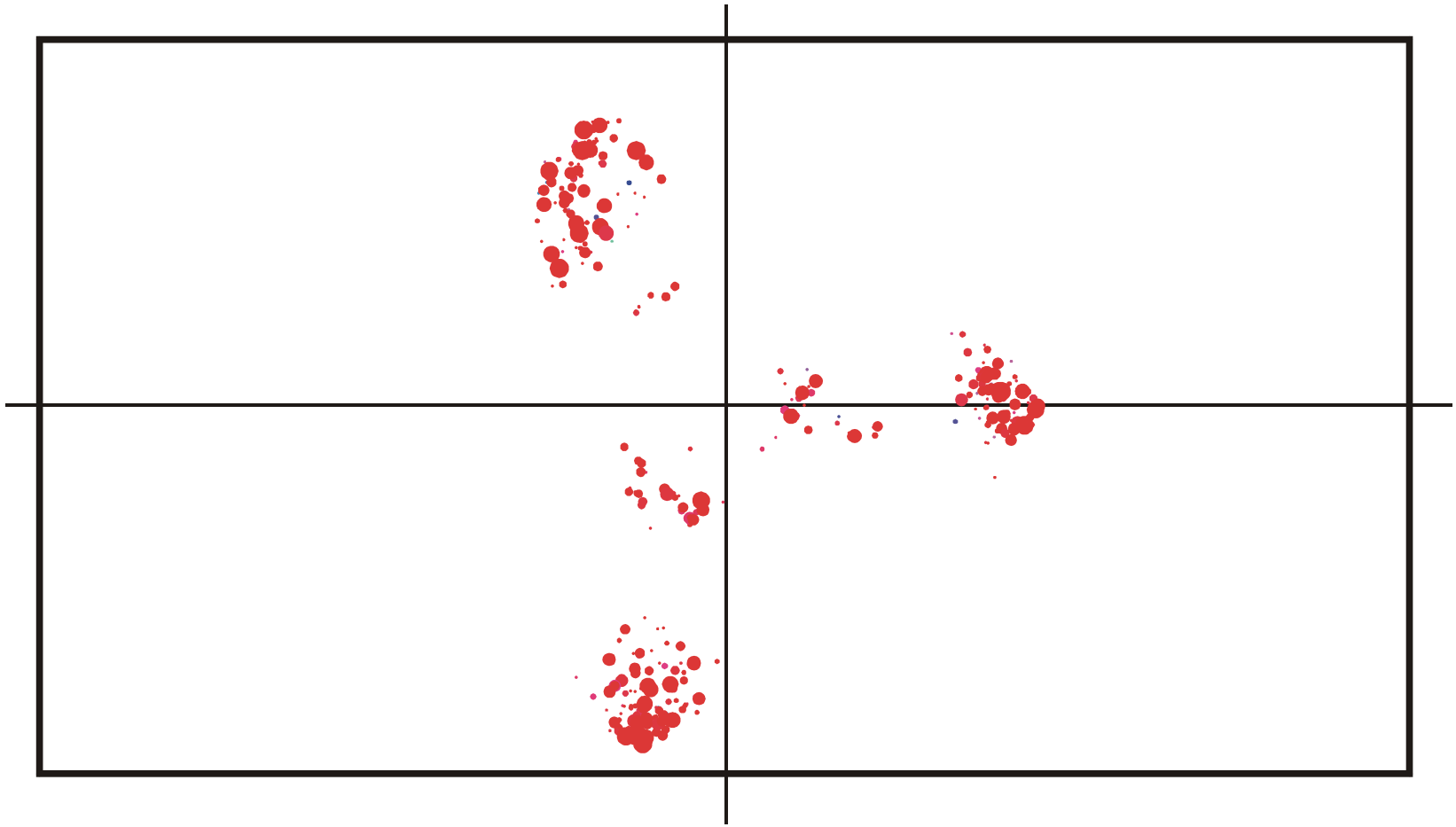
Spreading and evolution of a population on a neutral network : t = 170



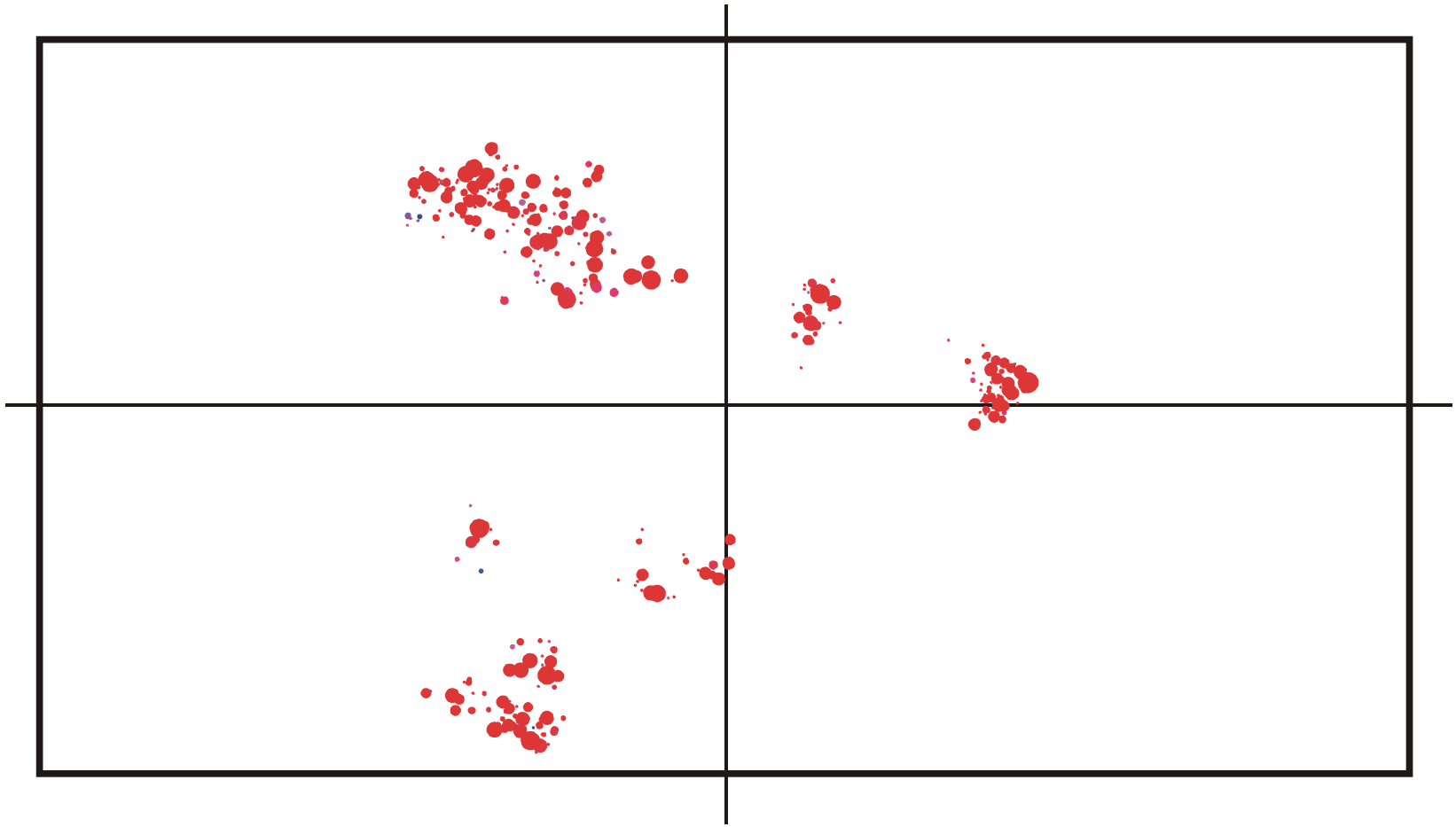
Spreading and evolution of a population on a neutral network : t = 200



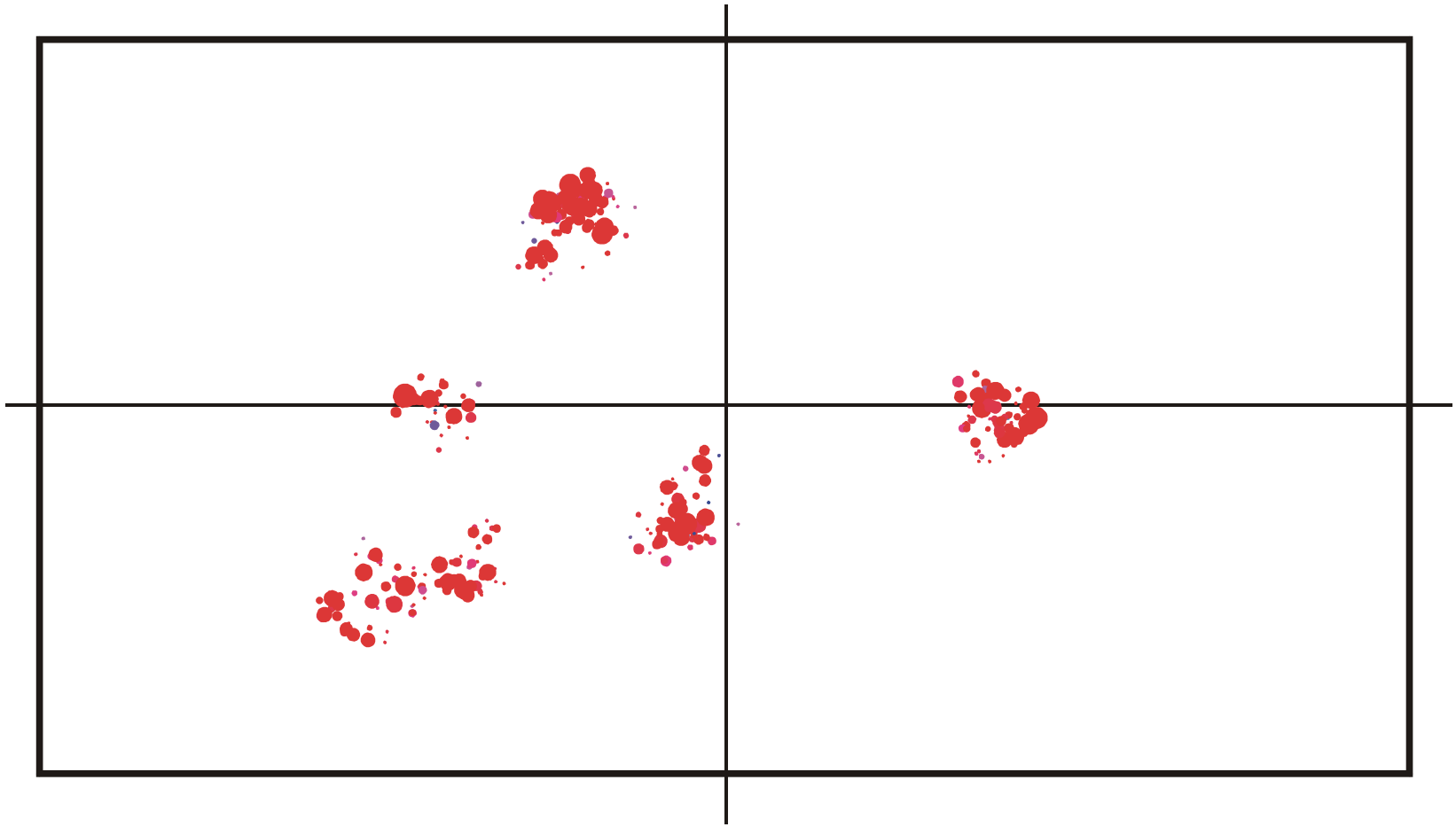
Spreading and evolution of a population on a neutral network : t = 350



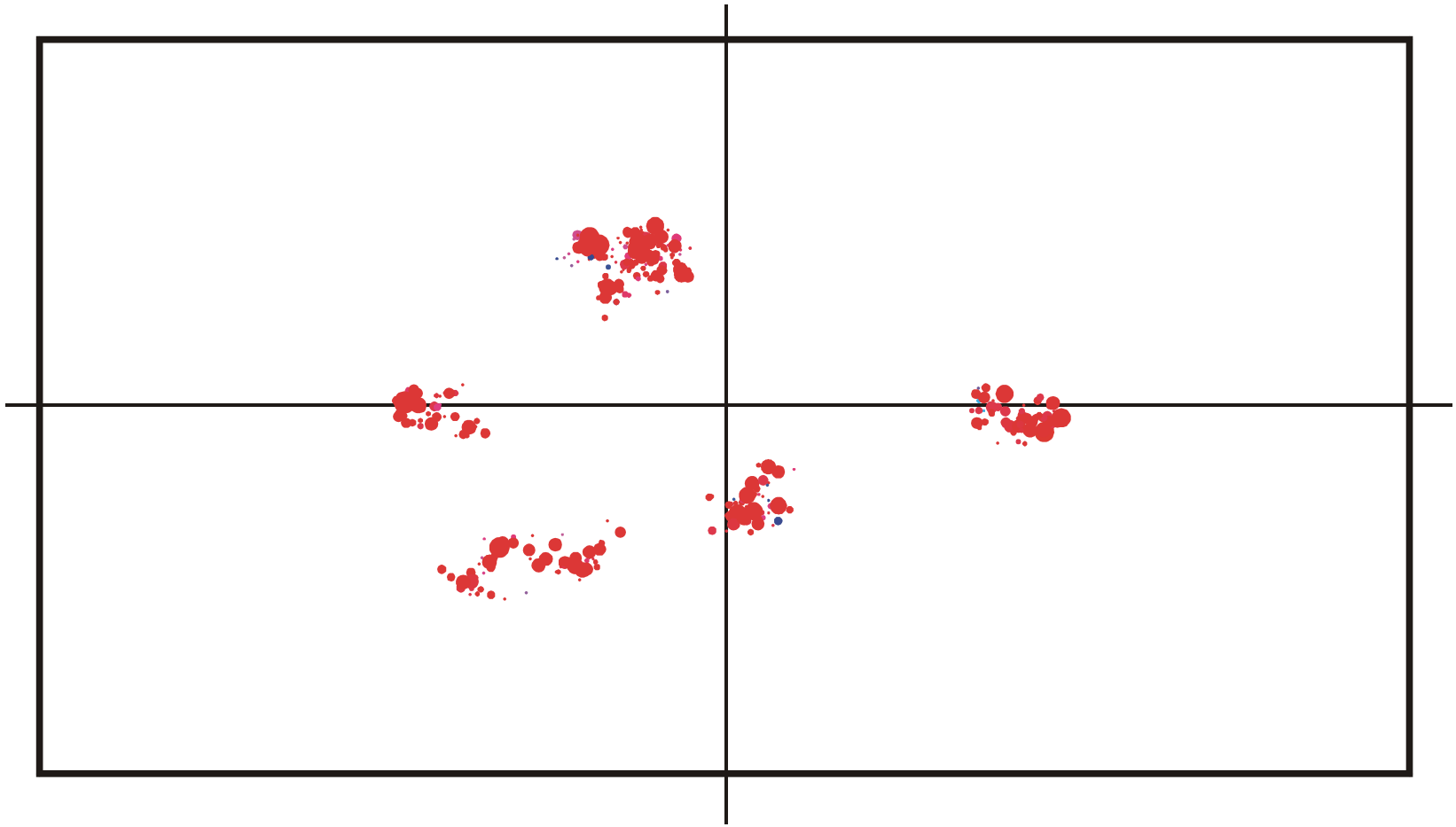
Spreading and evolution of a population on a neutral network : t = 500



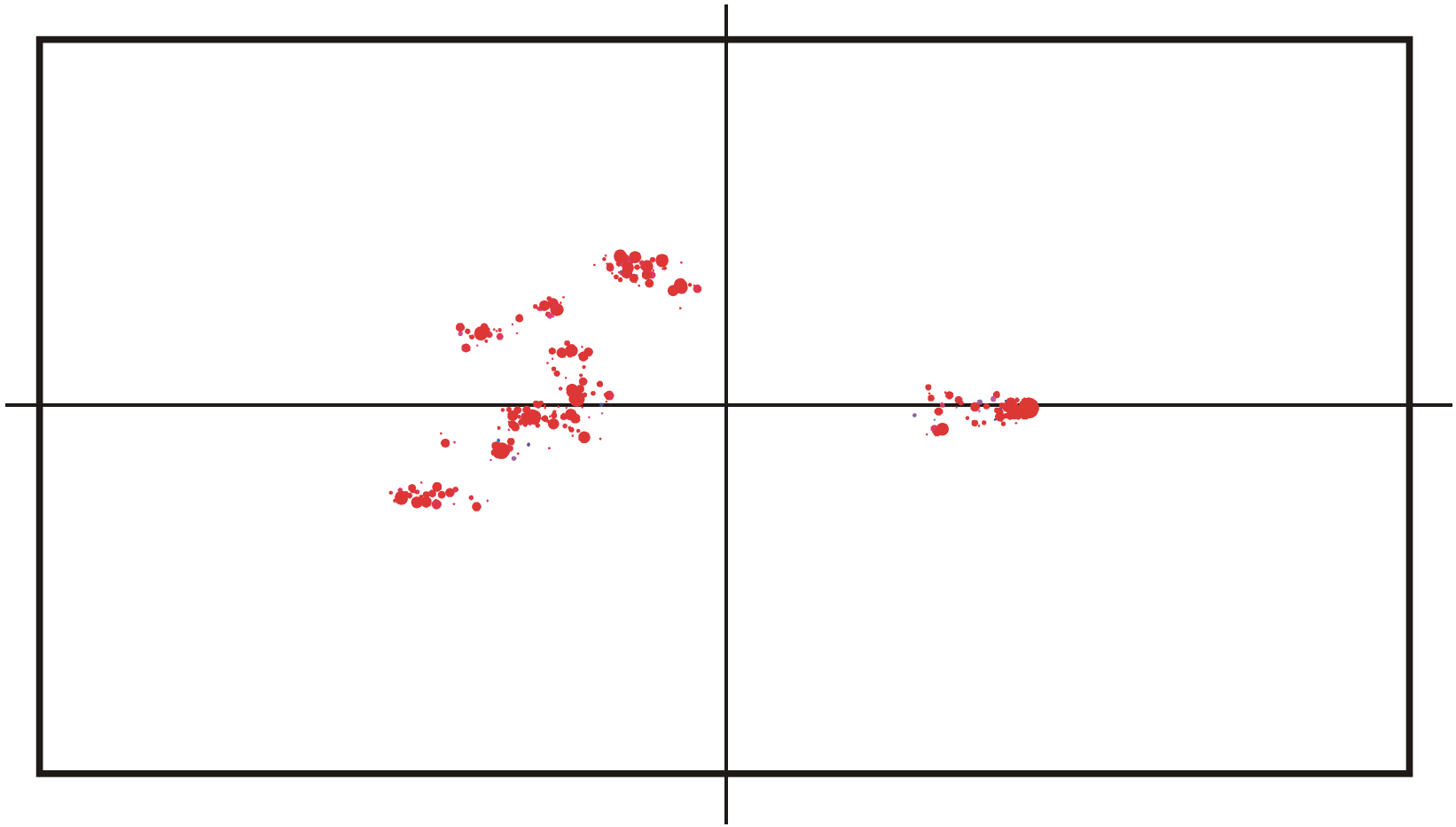
Spreading and evolution of a population on a neutral network : t = 650



Spreading and evolution of a population on a neutral network : t = 820



Spreading and evolution of a population on a neutral network : t = 825



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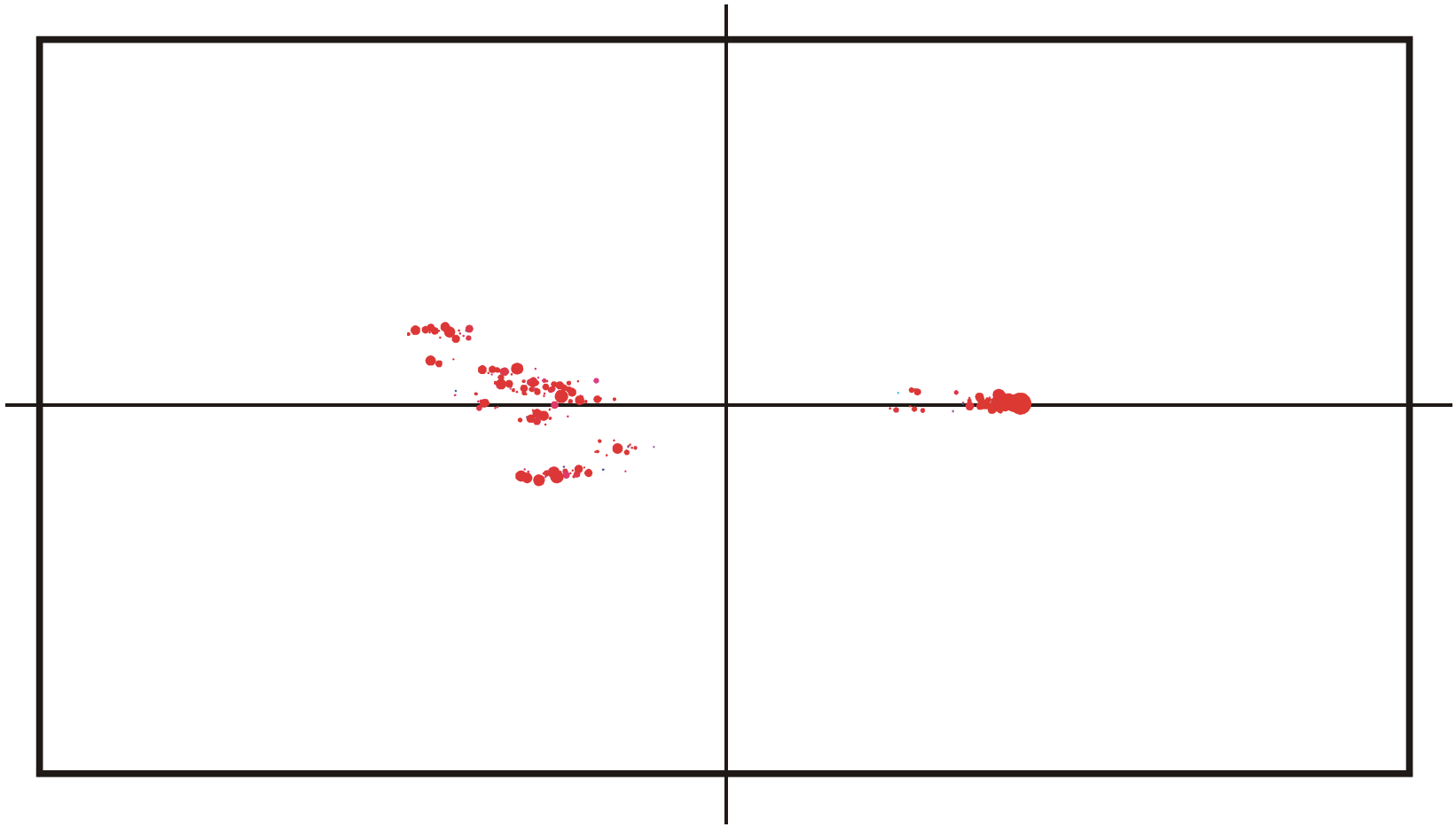

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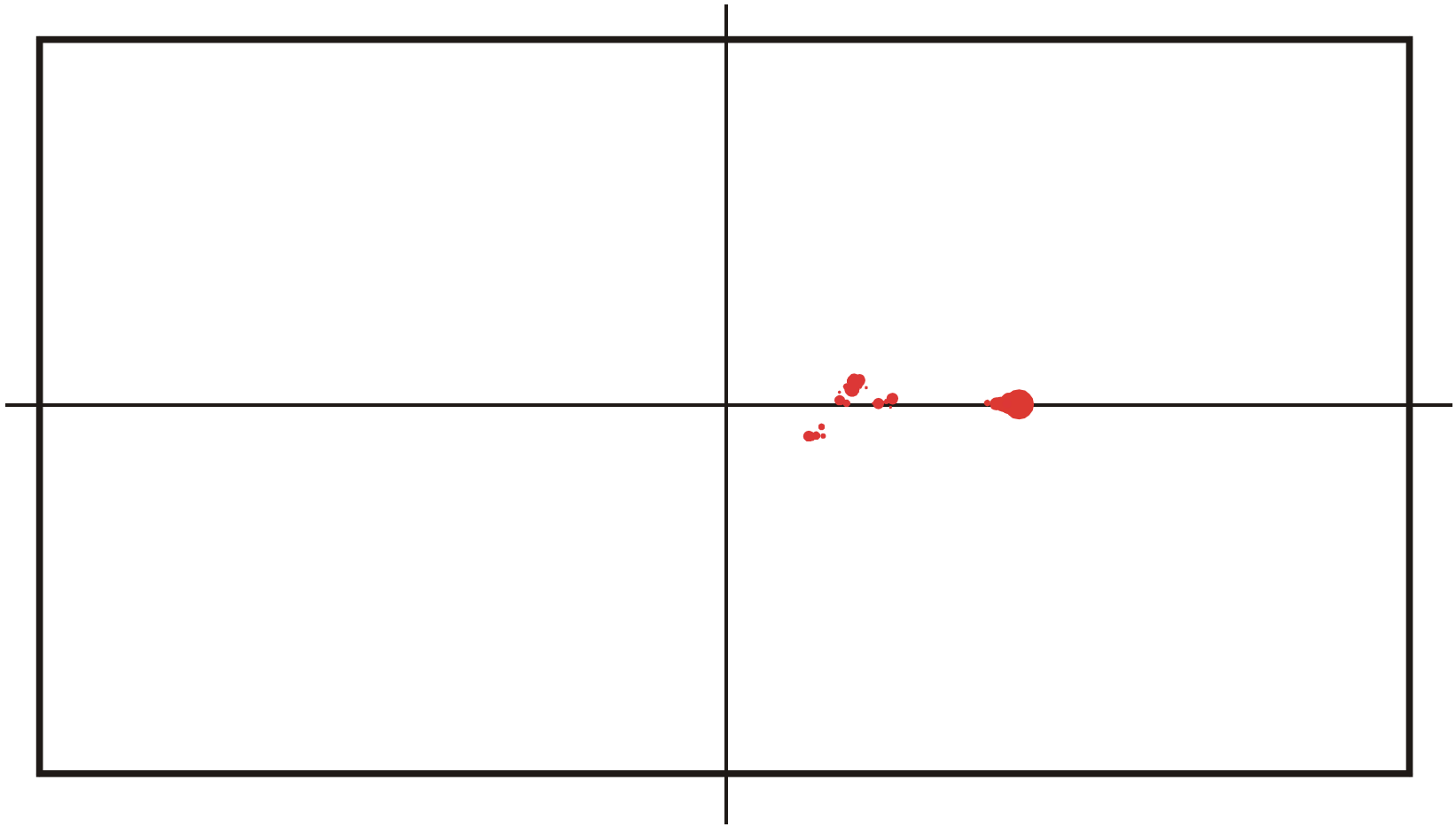
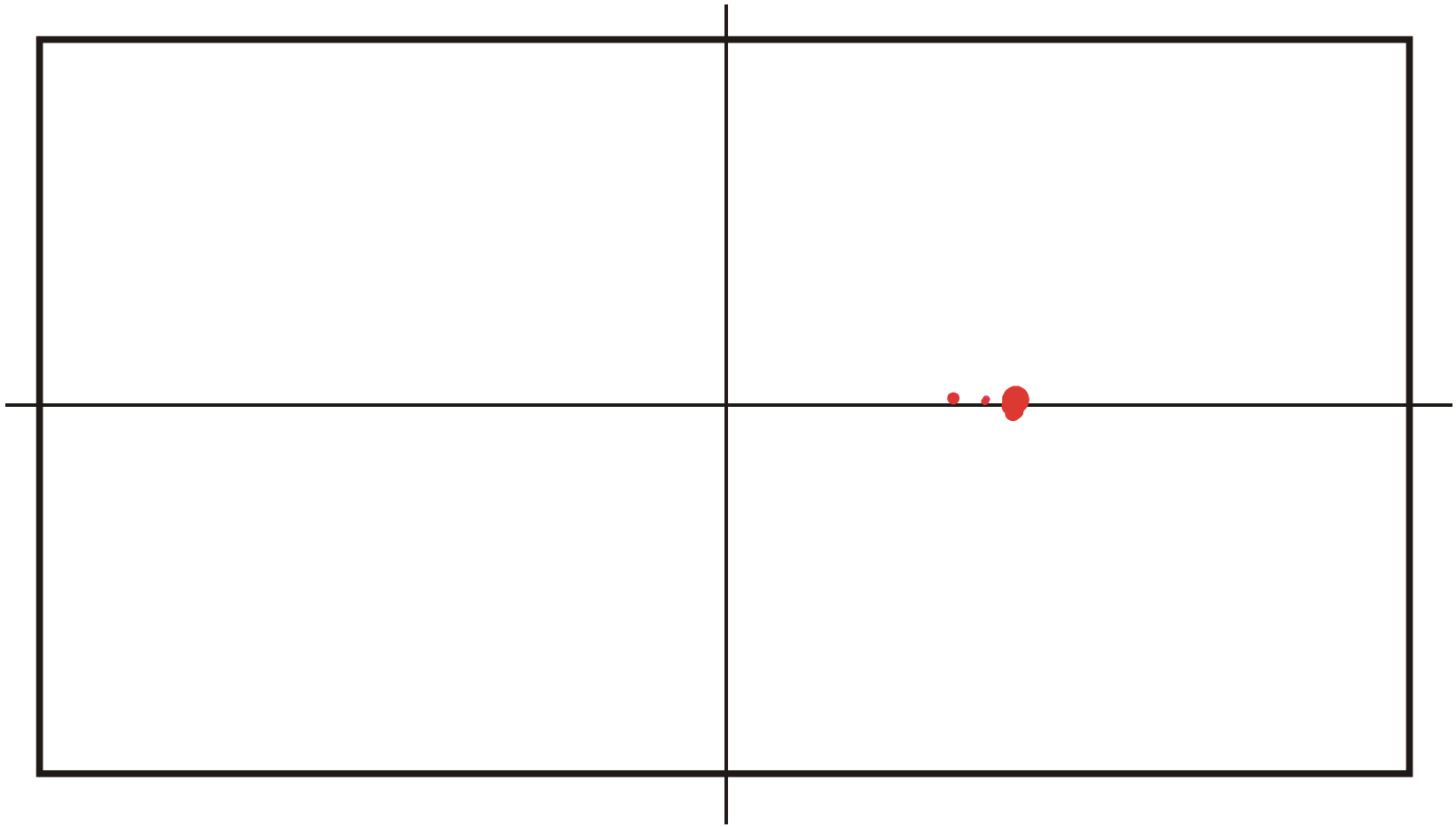
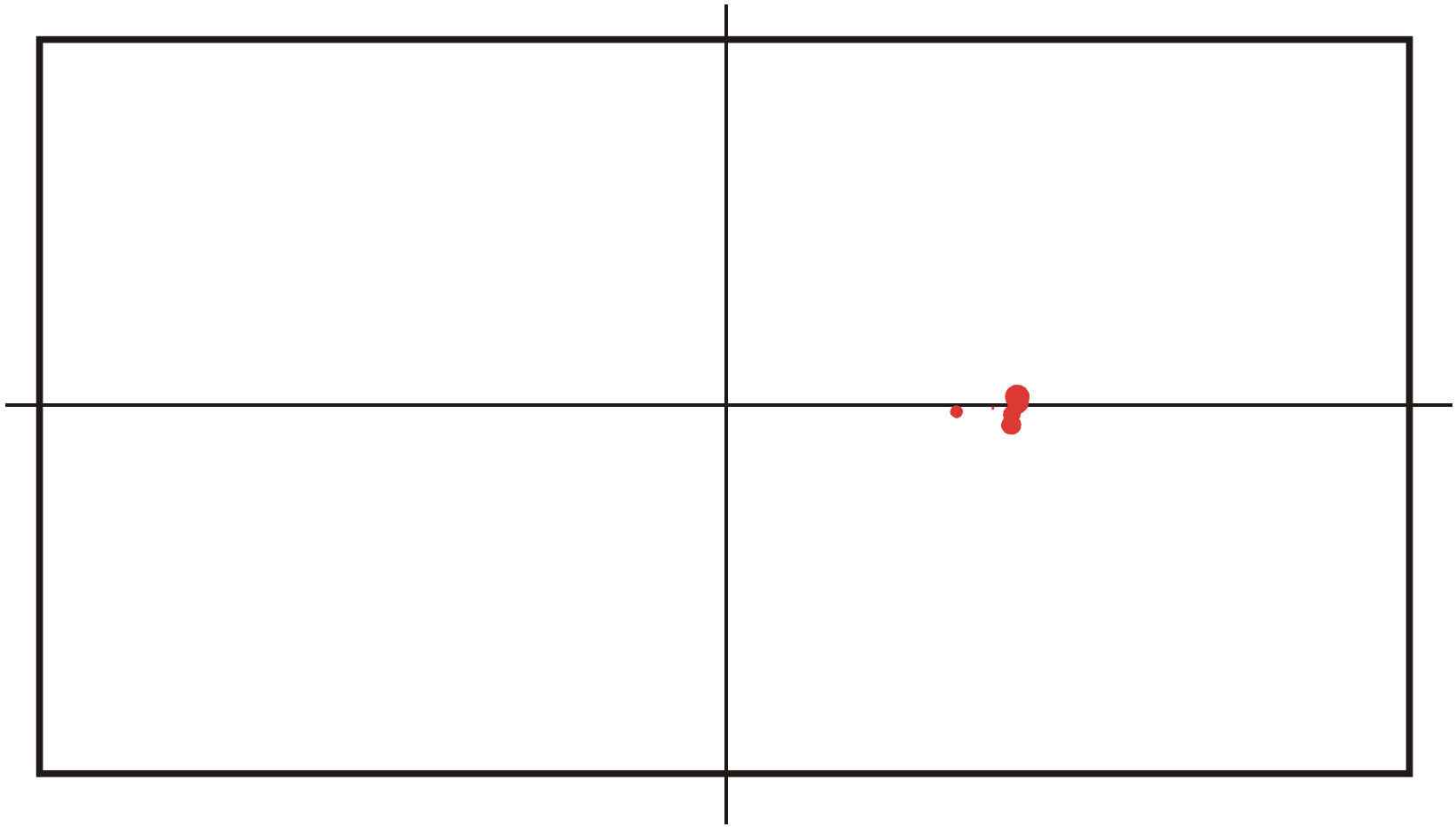


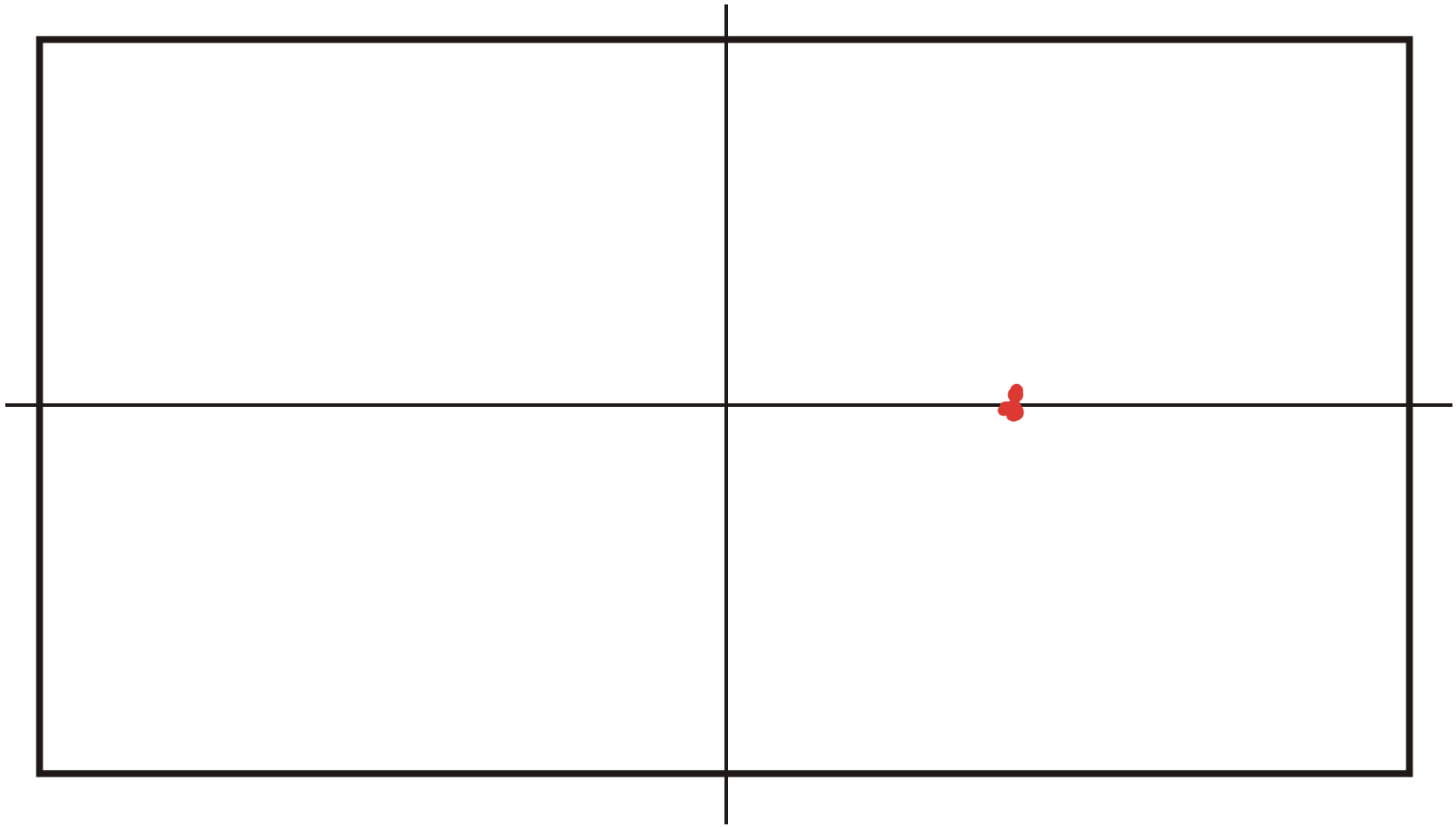
Spreading and evolution of a population on a neutral network : t = 840



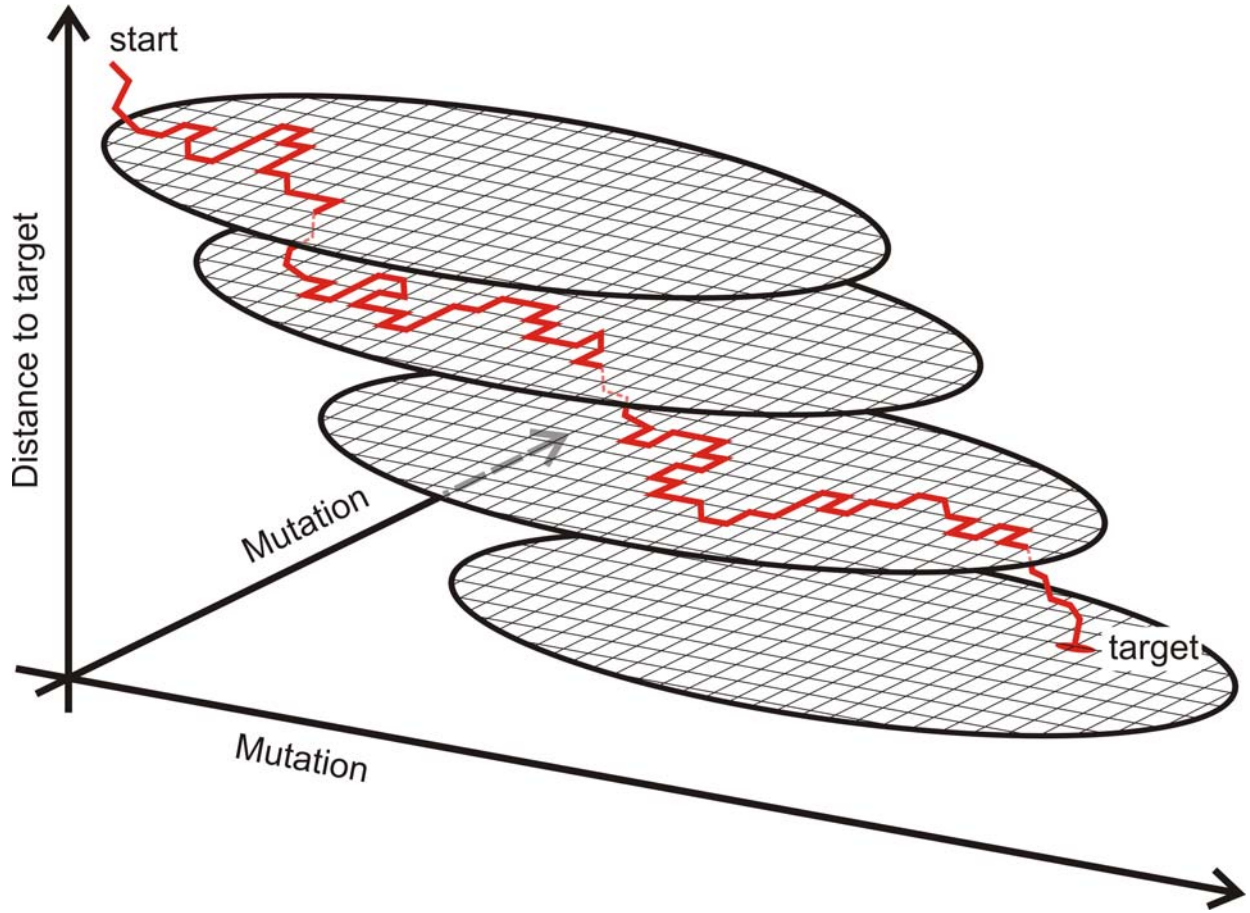
Spreading and evolution of a population on a neutral network : t = 845



Spreading and evolution of a population on a neutral network : t = 850



Spreading and evolution of a population on a neutral network : t = 855



A sketch of optimization on neutral networks

Table 8. Statistics of the optimization trajectories. The table shows the results of sampled evolutionary trajectories leading from a random initial structure, S_I, to the structure of $tRNA^{phe}$, S_T, as the target[a]. Simulations were performed with an algorithm introduced by Gillespie [55–57]. The time unit is here undefined. A mutation rate of $p = 0.001$ per site and replication were used. The mean and standard deviation were calculated under the assumption of a log-normal distribution that fits well the data of the simulations.

Alphabet	Population size, N	Number of runs, n_R	Real time from start to target		Number of replications [10^7]	
			Mean value	σ	Mean value	σ
AUGC	1 000	120	900	+1380 −542	1.2	+3.1 −0.9
	2 000	120	530	+880 −330	1.4	+3.6 −1.0
	3 000	1199	400	+670 −250	1.6	+4.4 −1.2
	10 000	120	190	+230 −100	2.3	+5.3 −1.6
	30 000	63	110	+97 −52	3.6	+6.7 −2.3
	100 000	18	62	+50 −28	–	–
GC	1 000	46	5160	+15700 −3890	–	–
	3 000	278	1910	+5180 −1460	7.4	+35.8 −6.1
	10 000	40	560	+1620 −420	–	–

[a] The structures S_I and S_T were used in the optimization:

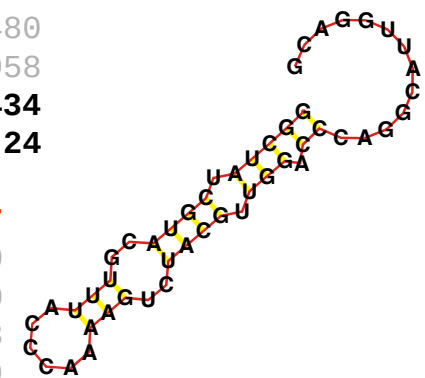
S_I: ((.((((((((((((.....((((....)))).....))))))).)))))))..))...(((.....)))
S_T: ((((((...((((........)))).((((.......)))))).....((((.......)))))..))))))....

Is the degree of neutrality in **GC** space much lower than in **AUGC** space ?

Statistics of RNA structure optimization: P. Schuster, Rep.Prog.Phys. 69:1419-1477, 2006

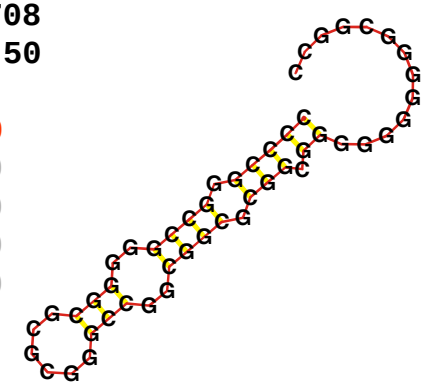
	Number	Mean Value	Variance	Std.Dev.
Total Hamming Distance:	150000	11.647973	23.140715	4.810480
Nonzero Hamming Distance:	99875	16.949991	30.757651	5.545958
Degree of Neutrality:	50125	**0.334167**	0.006961	**0.083434**
Number of Structures:	**1000**	**52.31**	85.30	**9.24**

1	(((((.((((..(((.....))))..)))))..)))).....	**50125**	**0.334167**
2	..(((.((((..(((.....))))..)))))..))).....	2856	0.019040
3	((((((((((..(((.....))))..)))))))).)).....	2799	0.018660
4	(((((.((((..((((....))))..)))))..)))).....	2417	0.016113
5	(((((.((((.((((.....))))..)))))..)))).....	2265	0.015100
6	(((((.(((((.(((.....))).)))))..)))).....	2233	0.014887



	Number	Mean Value	Variance	Std.Dev.
Total Hamming Distance:	50000	13.673580	10.795762	3.285691
Nonzero Hamming Distance:	45738	14.872054	10.821236	3.289565
Degree of Neutrality:	4262	**0.085240**	0.001824	**0.042708**
Number of Structures:	**1000**	**36.24**	6.27	**2.50**

1	(((((.((((..(((.....))))..)))))..)))).....	**4262**	**0.085240**
2	((((((((((..(((.....))))..)))))))).)).....	1940	0.038800
3	(((((.(((((.(((.....))).)))))..)))).....	1791	0.035820
4	(((((.((((.((((.....))))..)))))..)))).....	1752	0.035040
5	(((((.((((..((((....))))..)))))..)))).....	1423	0.028460



Shadow – Surrounding of an RNA structure in shape space – **AUGC** and **GC** alphabet

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

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