

range gravitational effects of the planets can lift the orbits of these objects beyond the outer edge of the planetary system, protecting them from close encounters with the planets. Therefore, many of these objects can survive in these eccentric orbits for the 4.6-billion-year age of the Solar System, forming a scattered disk.

Duncan and Levison's numerical simulations<sup>4</sup> indicate that objects in the scattered disk tend to have more eccentric and/or inclined orbits than those in the Kuiper belt. Two recently discovered trans-neptunian objects may be the first members of the scattered disk to be found. The first, 1996RQ<sub>20</sub>, was discovered in September 1996 by E. Helin, D. Brown and D. Rabinowitz. Its semi-major axis is 47 AU, its eccentricity is 0.3, its inclination is 32°, and it is about 300 km across<sup>13</sup>. The newly discovered second object, 1996TL<sub>66</sub>, is estimated<sup>3</sup> to have a semimajor axis of 84 AU, an eccentricity of 0.58, an inclination of 24°, and to be about 490 km across. These objects are clearly outside the range of orbital elements predicted for the Kuiper belt: 1996RQ<sub>20</sub> because of its inclination, and 1996TL<sub>66</sub> because of its eccentricity. However, they could be members of the scattered disk<sup>4</sup>.

With the recent advances in our observational and theoretical understanding of the trans-neptunian region, it is now possible to construct a tentative picture of how the outer Solar System formed and evolved.

Jupiter and Saturn formed early on, when the gaseous component of the solar nebula was still present. As the gas dissipated, it left behind a large number of small, icy bodies that had condensed from it, outside the orbit of Saturn and extending outward perhaps as far as a few hundred AU. These objects collided and gradually grew into larger 'planetesimals'. As the planetesimals increased in mass, they began to gravitationally perturb one another into more eccentric and more inclined orbits about the Sun. The growth of planets was much more rapid inside about 40 AU, leading to the formation of Uranus and Neptune. But outside 40 AU (that is, in the Kuiper belt), the bodies remained relatively small. Pluto may be just the largest Kuiper-belt object.

The young Uranus and Neptune scattered most of the remaining nearby planetesimals, outwards into the trans-neptunian region and inwards towards Jupiter and Saturn (Fig. 1). Usually, the objects scattered outwards eventually returned to the Uranus–Neptune region. In contrast, Jupiter and Saturn are so massive that they can efficiently eject many of these planetesimals out of the planetary system — so there was a net flux of objects towards Jupiter. This inward transport required an outward migration of the orbits of Uranus and Neptune in order to conserve the angular momentum and energy of the system<sup>14</sup>. As

Neptune moved outwards, it may have trapped planetesimals farther out into mean motion resonances<sup>10</sup>. Then Neptune's migration (and other processes<sup>11,12</sup>) would have pumped up the eccentricity of these objects to values as large as 0.3, leading to much of the present Kuiper-belt structure described above.

As mentioned, the objects scattered outwards by Uranus and Neptune can be long-lived. After their first encounter with Neptune, about 1% of them are stored in an extended scattered disk beyond Neptune for the age of the Solar System<sup>4</sup>. There are 31 Earth-masses of material in Uranus and Neptune, and as planet formation is far from 100% efficient, it is reasonable to expect that a similar amount of mass was initially scattered outwards by Neptune (the efficiency factor is not known to better than a factor of a couple of orders of magnitude). So, crudely, we may expect as much as a few tenths of Earth-masses of material in the scattered disk. This value is consistent with current estimates based on the dynamical models of the scattered disk by Duncan and Levison.

Much of the above picture is speculative, and much of it is likely to change rapidly in the coming years or months as this field

matures and more data become available. One reason for this is that only a very small fraction (about 0.06%) of the Kuiper-belt and scattered-disk objects have yet been discovered. But the fact remains that the structure of the trans-neptunian region is much more complex, and therefore interesting, than was dreamed of only a few years ago. □

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## Microbial genetics

# The tinkerer's evolving tool-box

E. Richard Moxon and David S. Thaler

The neo-darwinian synthesis resulted from the realization that mendelian inheritance changed the darwinian model of biological evolution. Darwin assumed that parents' genes blend into each other in their offspring. This was problematic because a fitter variant's genes would blend upon mating, and become diluted into the gene pool. Mendelism solved this paradox by providing a mechanism for descent, whereby individual alleles remain intact and are not blended away through mating. This example shows that knowledge of the specifics of genetics — that is, the mechanisms by which variants are generated — sometimes has a profound effect on evolutionary theory.

Another example of this is now provided by two papers on pages 700 and 703 of this issue. In a theoretical study, Taddei *et al.*<sup>1</sup> predict that in asexual, clonal populations of *Escherichia coli*, the ability to generate mutator alleles (which can lead to an increase in mutation rate) increases the rate at which more fit individuals arise in the population. This occurs even if the mutator alleles remain at a very low frequency. Sniegowski and colleagues<sup>2</sup> have found that three of 12 independently propagated clonal populations of *E. coli*, which were serially cultured over 10,000 generations and thereby subjected to alternating periods of growth and stasis, had a

mutator phenotype which was due to defects in genes involved in DNA repair.

These papers transcend the assumption that mutations are spread more or less evenly through a population: this assumption was only reasonable when mutation was considered to be a direct result of random insults from outside an organism. Mutations are now known to be due to processing of the internal consequences of such damage, as well as to endogenous processes. The internal processing — a part of DNA metabolism — is carried out by gene products whose alleles have profound effects on the generation of variation. Alleles that predispose other genes to (possibly beneficial) mutations may hitchhike<sup>3</sup>, because the mutator allele and the phenotypically selected alleles of other genes are linked, especially in asexual clonal populations.

Taddei and colleagues<sup>1</sup> detailed population analysis, derived using the best numerical parameters available for *E. coli*, shows that mutators are a key aspect of adaptive evolution. The implications of their results are strengthened by a previous theoretical model<sup>4</sup> showing that the effects of mutator alleles become larger as the evolutionary challenge is made more complex. The effect of incorporating mutators in the population increases exponentially as a function of the number of mutations required for a transition from one

local optimum in the evolutionary landscape to another, and how ill-adapted the intermediate genotypes are. There is an important difference between having the same number of mutations scattered randomly among individuals or, alternatively, having them clustered into a few highly mutating cells.

The inheritance of mutator alleles is not the only trick that bacteria have up their sleeve. Certain pathogens (viruses, bacteria and even large parasites) have subsets of genes that are excessively prone to mutation through, for example, slipped-strand mispairings, gene conversions and point mutations<sup>5</sup>. These hypermutable genes encode the surface molecules, such as adhesins or

invasins, that are involved in interactions with host molecules. Using just a few such loci, a population of microbes can use a combinatorial system (see box) to generate phenotypic variations which can influence many aspects of behaviour — antigenicity, motility, chemotaxis, attachment to host cells, resistance to desiccation, acquisition of nutrients and sensitivity to antibiotics. These hypermutable sequences have been called ‘contingency loci’ because they allow the pathogen to explore precipitous and unpredictable aspects of the host environment, while minimizing deleterious effects on fitness<sup>6</sup>. Duplications and recombination, both within and between genomes,

provide further options for combinatorial diversification<sup>7</sup>. Many of these diversity-generating mechanisms often act as a reversible binary switch.

In last week's issue, Bridges<sup>8</sup> discussed the physiological modulation of mutation rate<sup>9</sup>, and alluded to possible relationships of such mechanisms with the debate on the fundamental and controversial issue of directed mutation<sup>10</sup>. Are microbes smart enough to put two-and-two together, and coordinate physiological modulation of mutation rate with focused action at contingency loci? Both of the new papers<sup>1,2</sup> scrupulously avoid comment on directed mutation. It has been a tenet of molecular biology that genetic information is independent of events that occur outside, or even inside, the cell. The conventional view is that the environment selects among pre-existing variants — mutations arise without regard for their usefulness. But, intuitively, it seems advantageous for organisms to evolve mechanisms through which the environment could influence the genetic mechanisms that would alter the quantity and type of alleles in the repertoire on which selection acts.

The combination of contingency loci and physiological mutators goes some distance towards ‘directed mutations’, with no requirement for new molecular mechanisms or a reverse flow of information. The essentials of some of these ideas are touched upon in the ‘hypermutable state’ model proposed by Hall<sup>11</sup>. Contingency loci provide a molecular mechanism by which a hypermutable state is localized to particular regions of the genome. Physiological modulation of mutation implies that cells of increased fitness leave the mutator state and resume growth. Inheritance and mutation of the alleles of DNA metabolism may accomplish almost the same tricks as the physiological modulation of mutation rate, albeit more slowly and by a different evolutionary mechanism.

Whether or not the mutation rate is an important variable in bacterial pathogenesis, the presence of mutators in natural populations of organisms<sup>12</sup> must be considered in the context of resistance to antibiotics<sup>13</sup>. An analogous situation may apply to infection with human immunodeficiency virus<sup>14</sup>, where a high mutation rate is probably a key factor in the pathogenesis of disease progression. For many infections, the use of combination therapies (that is, simultaneous treatment with several drugs) is a favoured therapeutic strategy. If mutations can give rise to resistance, the rare clones that become resistant to several antibiotics are likely to be mutators. Cancer is also a ‘clonal pathogen’ — mutator alleles are oncogenes. This was predicted two decades ago<sup>15</sup>, based on the reasoning that the odds of several coincident mutations leading to a cancer in the same cell are too small at the normal mutation rate.

The generation of variation is itself under genetic control, allowing a reflectivity — an

## Algorithms for generating evolutionary variation

Pathogenic bacteria face particularly stringent tests of their adaptive potential because infections often occur within a short time frame (hours). During this crucial period, pathogens encounter the varying polymorphisms, immune repertoires and microenvironments of the host. In this race against time — with the escalation of host immune-clearance mechanisms and, perhaps, the administration of potent antimicrobials — the combinatorial effects of a minority subset of hypermutable (contingency) genes provides flexibility for adaptation.

Take the hypothetical genome of a pathogenic bacterium, comprising 2,000 genes including seven contingency genes (A–G), each of which can reversibly switch at a frequency of  $10^{-3}$  per bacterium per generation. Assume that each gene is a binary genetic switch (for example, A to A'), with corresponding phenotypes a or a' up to g or g'. If these genes switch independently, this gives 128 different phenotypic possibilities. Suppose that a bacterium of phenotype a, b, c, d', e, f, g is optimal for colonization of a host's epithelial cells, but long-

term colonization is favoured by entry into host cells. Now suppose that bacteria with the phenotype a', b, c, d, e, f', g' (involving four switches from the original) are best able to invade and survive in the cells of the host: these bacteria will be selected as the adaptive phenotype. This is a rare event — about one in a trillion cells. But a population of cells with a 100-fold increase in mutation rate will, on average, contain an individual of the requisite genotype when the population size is only 10,000. This phenotypic variation, which is

stochastic with respect to the timing of switching but has a programmed genomic location, allows a large repertoire of phenotypic solutions to be explored, while minimizing deleterious effects on fitness.

The combinatorial strategy exploited by contingency loci is found elsewhere, generating the diversity seen by Mendel and in the influenza virus. Jacob<sup>19</sup> spoke of “The mode of operation of the tinkerer... arranging various combinations to produce new objects of increasing complexity”. The algorithm (tool-box) of the tinkerer also evolves:

### The tinkerer's tool-box

Genetic determination of mutation rate by standard genes of DNA metabolism

- Polymerase editing
- Post-replication mismatch correction, mutS, mutL, mutH, mutU
- Chemical lesion processing

Physiological modulation of the generation of variation

- SOS
- Stringent response
- Carbon starvation
- Transient mutators
- Meiosis

Focusing change differentially in the genome

- Contingency loci
- Gyrase binding sites
- Duplication, deletion or inversion between repeats
- Site-specific recombination
- Hot and cold regions for generalized recombination

informational feedback — on the mechanisms by which diversity is generated in biological evolution<sup>4,16,17</sup>. As E. G. Leigh<sup>18</sup> wrote: “selection will hold mutators fully responsible, as it were, for both the good and the bad they cause”. The implications of this evolving feedback are of significance to biologists, mathematicians and others concerned with information flow in dynamical systems. □

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Origins of life

# The first two billion years

Eörs Szathmáry

The problems associated with defining the origins of, and transitions between, different levels of biological organization receive increased attention these days, and rightly so. New discoveries and models help us to account for the processes that resulted in new ways of storing and retrieving hereditary information. These processes often relied on the formation of higher-level evolutionary units from previously unlinked replicators — small units that can replicate independently<sup>1</sup>. This was the subject of a recent meeting<sup>\*</sup>, organized by the Swedish Natural Science Research Council, at which topics ranging from the origin of the first replicators to the emergence of multicellular eukaryotes were discussed.

The origin of non-enzymatic replication is still an unsolved problem. Although artificial replicators can grow without a replication enzyme (replicase) in a test tube<sup>2</sup>, they are not a historically realistic recapitulation of what could have happened on the early Earth. But there are three promising lines of research: replication of nucleic acids on a surface; *in vitro* construction of a catalytic RNA (ribozyme), which could act as a generalized replicase; and formation of oligonucleotides by ligating together smaller nucleic-acid units.

Surface-bound template polymerization has been successful up to a length of 55 nucleotides<sup>3</sup>. At this length, ribozymic activity of an RNA sequence is also feasible. An RNA-dependent RNA polymerase ribozyme can copy templates of up to six nucleotides with remarkable efficiency<sup>4</sup>, although the ultimate aim would be to have a molecule that could copy any sequence, including

itself. Replicase ribozymes would be especially useful in studying autonomously evolving ‘ecosystems’ of molecules. For example, A. Eschenmoser (ETH, Zürich) asserts that pyranosyl-RNA (pRNA) is a possible substitute for the commonly occurring furanosyl variant (Fig. 1a): pRNA has a stronger and more selective base-pairing system than ordinary RNA. So why did nature first, maybe, choose pRNA, and why was it later abandoned?

Tetranucleotide-2',3'-cyclophosphates have also recently been shown to assemble

into oligomers of up to 36 nucleotides in dilute solution<sup>5</sup> (Fig. 1b). This oligomerization is highly chiroselective — a homochiral template catalyses the formation of the correct phosphodiester junction between homochiral tetramers that have the same ‘handedness’ as the template.

The origin of biomolecular homochirality is another important aspect, because it is connected to the notion of replicators with limited or unlimited heredity<sup>1,6</sup>. For replicators that have a limited ability to pass on characteristics, the number of individuals will typically exceed the number of possible sequences — an example is a replicating oligonucleotide. Contemporary nucleic-acid-based replicators in living systems have, in contrast, unlimited heredity, and a museum showing all of the possible sequences up to a certain length would be larger than the Universe.

Imagine that one starts with a mixture of homochiral templates of D- and L-enantiomers, and the degree of oligomerization grows with time: each chiral template gives rise to its own kind. Eventually, the supply of raw materials will be exhausted, and it will no longer be possible to have at least one copy of each sequence. Some sequences will be present in only one enantiomeric form or the other, so the symmetry of the molecular population will be spontaneously broken, paving the way for the passage to unlimited heredity. One population will now become more successful than the other, because it will consist of fitter sequences; and, ultimately, the population with the opposite sense of chirality will be competitively displaced<sup>5</sup>.

But what about the origin of homochiral tetramers, or enantiomeric excesses in gen-

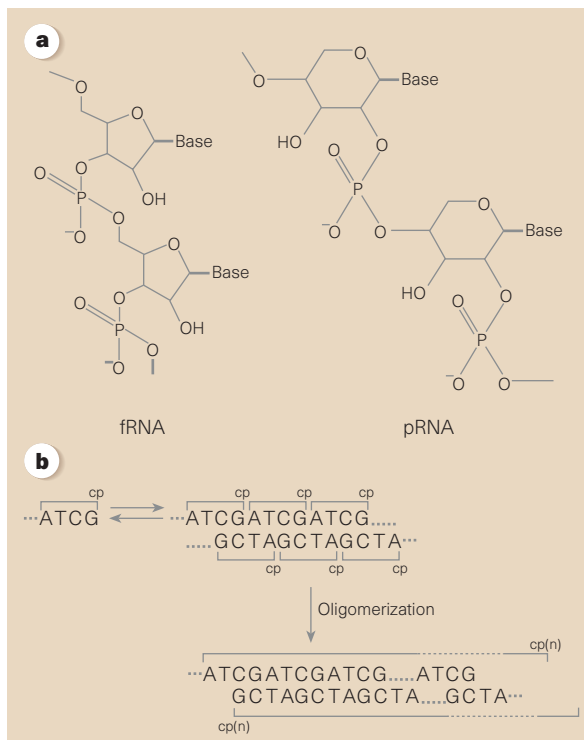


Figure 1 The origins of nucleic-acid-like molecules. a, The common (furanosyl) form of RNA (fRNA) may have superseded the pyranosyl form (pRNA), in spite of the fact that pRNA has a stronger and more selective base-pairing system. b, In dilute solution, tetranucleotide cyclophosphates (cp) can assemble into oligomers up to 36 nucleotides in length. The oligomerization is chiroselective, so only tetramers that have the same chirality as the template are joined — when one of the (D)-ribopyranosyl units at any position of a homochiral (D)-tetramer is replaced by a corresponding (L)-unit, the rate of oligomerization is reduced by about two orders of magnitude.

\* Scientific Forum on the Origin of Life, Arken, Gothenburg, Sweden, 7–9 April 1997.