make sure they respond uniformly) and then irradiated with polarization-shaped pulses. These molecules — iodine[1] and potassium[2] —are as simple as molecules can be, but they are already too complex to theoretically design an optimized polarization structure that would maximize ionization. Therefore, both experiments relied on self-learning techniques, in which the optimal pulse shape and polarization structure (Fig. 1) are found through an iterative optimization procedure. Both groups have shown conclusively that pulses with complex polarization structures ionize these molecules more efficiently than pulses with a uniform polarization.

Further work will surely follow, using polarization-shaped pulses to tweak atomic and molecular systems with greater precision. Several proposals have already been made and await experimental tests, including the alignment of molecules in a gas phase, the manipulation of chiral molecules and the control of attosecond pulse formation. Expect laser scientists to steer towards even more uses for these twisted pulses of light. ■

Yaron Silberberg is in the Department of Physics of Complex Systems, Weizmann Institute of Science, Rehovot 76100, Israel.
e-mail: yaron.silberberg@weizmann.ac.il

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Genomes

Worming into genetic instability

Susan M. Rosenberg and P. J. Hastings

A study of roundworms shows that genomic mutations occur surprisingly frequently, and that the kinds of changes involved differ from those predicted. Are genomes inherently less stable than previously suspected?

DNA carries the coded information that specifies the size, shape, body plan and many other basic characteristics of most organisms. To transmit these characteristics faithfully, DNA must pass from generation to generation with relatively few mutations. But mutations do happen, and can have profound consequences. These include inherited diseases, cancer and drug-resistant infections, but also the genetic differences among individuals that, through natural selection, drive evolution.

Until now, mutations seemed to be relatively rare and to occur in a characteristic spectrum. But such observations are challenged in the paper by Denver and colleagues on page 679 of this issue[1]. These authors used a particularly powerful way to hunt for mutations in the roundworm *Caenorhabditis elegans* (Fig. 1, overleaf) — and found at least ten times more mutations, and a different assortment, than anticipated.

Traditional ways of estimating mutations are indirect[2], involving either phylogenetic studies of wild organisms or phenotypic methods in the lab. Phylogenetic studies involve comparing DNA sequences between species and estimating the number and kinds of changes that have occurred since the species diverged. Phenotypic methods rely on the ability of some mutations to change a trait (phenotype) of an organism. After a defined number of generations, rare mutants carrying the new trait are quantified, and mutation rates are calculated and then extrapolated to predict rates for the whole genome. This extrapolation takes into account the genome's size and the fraction of mutations that has been estimated to produce phenotypic change (about one-third)[2].

However, both approaches probably underestimate the inherent mutation rate and skew the variety of mutations found. For instance, some mutations are harmful, and so the organisms that carry them are less likely to contribute to the next generation (they are 'selected against'), both in the wild and in large cultures. And the fraction of mutations that produces no phenotypic change might be larger than imagined.

Denver *et al.*[1] bypassed the phenotype-bias problem by directly sequencing randomly chosen stretches of DNA in laboratory-grown worms. They also minimized selection against harmful mutations by maintaining many lines of worms, separating a single worm from each progeny and allowing it to produce the next generation by self-fertilization, without competing with other worms. Rapid and severe loss of fitness occurs in these worms because, when their numbers are reduced to one repeatedly, random mutations become fixed — a phenomenon known as Muller's ratchet[3].

From these pampered worms, Denver *et al.* sequenced four million base pairs of DNA, and found 30 new mutations compared with the original animals. This equates to a rate of 2.1 mutations per genome per generation. This rate is at least ten times higher than those reported previously in worms and other DNA-based organisms,

100 YEARS AGO

Prof. Schäfer, F.R.S., describes a simple and efficient method of performing artificial respiration in the human subject, especially in cases of drowning… Immediately the patient is recovered from the water he is placed face downwards, the head being turned sideways so that the mouth and nose are unobstructed, with a folded coat under the lower part of the chest; if respiration has ceased every instant of delay is serious. The operator then places himself athwart, or on one side of, the patient's body in a kneeling posture and facing the head. He places his hands flat over the lower part of the back (on the lowest ribs), one on each side, and gradually throws the weight of his body on to them so as to produce firm pressure — which must not be violent — on the patient's chest. This compresses the chest, and air (and water if there be any) is driven out of the patient's lungs. He then raises his body slowly so as to remove the pressure, still keeping his hands in position. This process of applying pressure and of relaxation of pressure by the forward and backward movement of the operator's body is repeated every four or five seconds without any marked pause between the movements. This course must be pursued for at least half an hour, or until the natural respirations are resumed… If there be means, others may remove the wet clothing by cutting it off, and may apply hot flannels to the body and limbs and hot bottles to the feet.
From *Nature* 4 August 1904.

50 YEARS AGO

The United Kingdom Atomic Energy Authority has announced that a heavy-water reactor (or atomic pile) which has been built at the Atomic Energy Research Establishment, Harwell, is now in operation. 'Dimple' is a low-powered thermal neutron research reactor. The heavy-water moderator is contained in a tank which is surrounded by a graphite neutron reflector. Outside this is a concrete radiation shield. The reactor fuel is submerged in the heavy water. Both the type of fuel and its arrangement in the tank can be changed quickly so that what is, in effect, a different design of reactor can be built up in a matter of days… The versatility of Dimple will make it an extremely valuable tool in the design of future power-producing reactors and for measuring essential constants in reactor physics.
From *Nature* 7 August 1954.

J. KING-HOLMES/SPL

Figure 1 **The roundworm — lessons in mutation.**

which curiously maintain constant predicted mutation rates per genome, irrespective of genome size[2]. Moreover, the kinds of mutations differ from those previously seen in many organisms, even worms. Why so many and such different mutations?

Denver *et al.* suggest that the crucial difference lies in reducing the genetic selection that biases the accumulation of mutations. Specifically, they find that insertions of one to a few bases of DNA are more common than previously reported, and argue that these findings, when compared with the results of phylogenetic studies, suggest that larger genomes are selected against in the wild. This might be true. But Denver *et al.* also found more insertions than are seen in lab-based studies[2], in which selection for small genome size should be minimal.

We suggest two other explanations. First, previous estimates of how many mutations give rise to phenotypic changes might be too low. Phenotypes might be masked by variables not factored in, such that mutation rates are higher than predicted from lab-based data — as Denver *et al.* observe. For example, phenotypes of altered proteins are known to be masked, or 'buffered', by molecular chaperones, molecules that spruce up distorted proteins into better approximations of their functional shapes. Loss of available chaperones — in response to 'heat shock', for instance — reveals many new phenotypes, produced by previous mutations, that are invisible with chaperones present[4]. Also, the numbers and kinds of mutations to which specific DNA sequences are prone might be an evolved trait[5]; perhaps most genes are actually less prone to mutations that cause phenotypic changes. So, correction factors for the number of genomic mutations per phenotypically detectable mutation may need to be revised upward. Denver and colleagues' data could help to provide an empirical correction factor.

A second, not mutually exclusive idea is that a state of increased mutability (a 'mutator' state) is induced in the worms specifically because they have accumulated harmful mutations through Muller's ratchet. We suggest that these mutations provoke cellular stress responses that, in turn, cause further mutations. The idea that Muller's ratchet can provoke general stress responses is supported by studies of the bacterium *Buchnera*, which endures severe population 'bottlenecks' and induces the production of a protein involved in the heat-shock/protein-stress response[6]. And in yeast, most gene-inactivating single mutations cause decreased fitness and altered patterns of expression of other genes[7], perhaps reflecting the activation of stress responses.

Furthermore, at least two stress responses have been documented to cause mutations: the so-called SOS response to DNA damage in bacteria[8]; and the bacterial general-stress response, controlled by the RpoS protein[9,10], which increases the activity of some 50 genes in response to starvation and to temperature, pH, osmotic and oxidative stresses. One of these genes encodes a DNA-synthesizing enzyme[11] whose error-prone nature might underlie some of the mutation-inducing effects of this stress response[9,10]. Moreover, as mentioned above, the heat-shock stress response exposes phenotypic variation[4] — but might also cause genetic variation by increasing the mutation rate. We predict that the heat-shock response will indeed be found to cause mutations, and that many other general-stress responses will do so too.

It makes sense for stress responses to cause mutations; it may be a 'selected' feature that increases genetic variation, thus increasing 'evolvability' under stress when organisms are suboptimally adapted to their environments. Most of the mutations would be harmful or neutral, but rare adaptive mutations would also occur. Rare individuals in large stressed populations could thereby flourish, then, later, turn off their stress responses and readjust their mutation rates downwards to achieve greater genetic stability. Mutation-inducing stress responses might also underlie most cancers, which do not acquire mutator mutations, but still accumulate surprisingly high numbers of tumour-promoting alterations. Curiously, non-mutational gene-silencing events are common early in tumour progression, whereas mutations are more common later[12]. Perhaps this is because early gene-inactivating events cause mutation-inducing stress responses. Denver and colleagues' method and findings may provide windows on both of these important processes.

The nature of the alterations found by Denver *et al.*[1] supports the idea of a connection between mutations and stress responses. They strikingly resemble the mutations seen in cells lacking DNA 'mismatch repair'[13], which corrects replication errors and is debilitated during a mutational stress response. Both the worms and mismatch-repair-defective cells show 10–100 times more mutations than normal worms or cells; more small sequence insertions and deletions than base substitutions; more insertions and deletions in single-base repeats; and more 'transitions' than 'transversions' among base substitutions. We suspect that loss of mismatch-repair capacity is a general feature of mutation-promoting stress responses, as observed in starved bacteria[14], in which a special error-prone DNA polymerase enzyme, induced by the SOS response[8] and starvation[11], causes increased mutation[15]. In general, whenever excessive replication errors occur, mismatch repair should become exhausted[14–16]. So Denver and colleagues' creatures may have wormed their way into genetic instability. Whatever the mechanism that accounts for their altered DNA, they are telling us something important about mutation. ■

Susan M. Rosenberg and P. J. Hastings are in the Department of Molecular and Human Genetics, and Susan M. Rosenberg is also in the Departments of Biochemistry and Molecular Biology, and Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas 77030-3411, USA.
e-mail: smr@bcm.tmc.edu

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