therefore seems at least qualitatively consistent with the observed poleward migration of annual-mean LMI.

Despite the large and statistically significant global trends in the annual-mean latitude of LMI, substantial region-to-region and year-to-year variability is evident. For instance, the North Atlantic region, which has received considerable media attention owing to events such as hurricanes Katrina and Sandy, shows almost no poleward trend on the basis of historical 'best-track' data over the past 30 years. Moreover, when the authors used a state-of-the-art data set of tropical-cyclone intensity (ADT-HURSAT; ref. 5), an opposite, equatorward, trend is found for the North Atlantic (see Table 1 of the paper[2]). Such regional differences in trends are probably due to climate modes that extend in time beyond the period for which accurate satellite-based data are available.

This is one of the limitations of trend studies based on satellite-derived estimates of tropical-cyclone intensity. Although the post-1970s geostationary satellite era is considered to be the most accurate part of the historical tropical-cyclone record, the relatively short observation period hampers the detection of trends influenced by modes of climate variability whose periodicity spans decades or longer, such as the Pacific Decadal Oscillation[6]. Any such variability implies that regions in which the poleward migration of annual-mean LMI has been more pronounced over the past 30 years might experience less-pronounced trends in the coming decades, and vice versa. Even on a global scale, a trend of 1° of latitude per decade of tropical expansion (that is, a 10° shift per century, assuming a constant rate of expansion) cannot be sustained without implausible changes to fundamental physical constraints on the global atmospheric circulation, such as Earth's rotation rate.

On year-to-year timescales, variability in tropical-cyclone formation and track is dominated by the phase of the El Niño–Southern Oscillation (ENSO) — the episodic warming (El Niño) and cooling (La Niña) of the surface temperature of the tropical Pacific Ocean. El Niño often promotes an equatorward migration of tropical-cyclone activity, whereas during La Niña a poleward displacement is observed[7], concomitant with changes in the width and intensity of the Hadley circulation[8]. It is therefore plausible that any trend in ENSO could project onto trends in tropical-cyclone activity. Kossin *et al.* attempt to remove this contribution by accounting for the effect of ENSO on the linear trend of annual-mean LMI latitude and then examining the residual data. The poleward migration remains pronounced and statistically significant, suggesting that ENSO plays only a minor part in the long-term hemispheric and global trends.

Kossin and colleagues' findings provide insight into the response of global tropical-cyclone activity to a changing climate. However, several questions remain unanswered. For instance, will future changes in wind patterns cause storms to move towards or away from coastlines[9]? What are the key mechanisms driving the observed tropical expansion, and how do these tie in with factors known to modulate tropical-cyclone intensity? Such questions remain the subject of future research. ■

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SYNTHETIC BIOLOGY

New letters for life's alphabet

The five bases found in nucleic acids define the 'alphabet' used to encode life on Earth. The construction of an organism that stably propagates an unnatural DNA base pair redefines this fundamental feature of life. SEE LETTER P.385

ROSS THYER & JARED ELLEFSON

All known life forms store and transmit information from generation to generation using the bases found in nucleic acids: adenine, cytosine, guanine, thymine and uracil. In nucleic-acid double helices, these form base pairs (guanine with cytosine, and either adenine with thymine in DNA, or adenine with uracil in RNA), which are mostly orthogonal — that is, little pairing occurs between other combinations of bases. However, this 'alphabet' seems to be an accident of history rather than a functional necessity, given that other orthogonal base pairs have been synthesized and shown to be processed by DNA-replication enzymes *in vitro*[1]. Because life on Earth is biochemically uniform, the formal possibility of alternative alphabets requires strong experimental proof. In this issue, Malyshev *et al.*[2] (page 385) provide just such a proof, by conclusively showing that an unnatural base pair can be stably propagated in the bacterium *Escherichia coli*.

Shortly after the discovery of DNA, it was proposed[3] that analogues of natural bases could form a third functional pair, but nearly 30 years passed before advances in organic synthesis and the development of methods for amplifying DNA gave scientists free reign to explore this hypothesis. In 1989, a base pair formed from isomers of guanine and cytosine was synthesized, and replication, transcription and even translation of DNA sequences incorporating this base pair were demonstrated *in vitro*[1,4]. Then in 1995 came the surprising finding[5] that hydrogen bonding between bases was not an absolute requirement for complementary binding, and could be replaced by steric compatibility (the fitting together of matching molecular shapes) and hydrophobic interactions. This culminated in the independent development of three highly orthogonal base pairs[6–8], each capable of *in vitro* replication fidelity exceeding 99%.

Malyshev *et al.* now describe the development of a bacterium capable of faithfully replicating a plasmid — a small, circular DNA molecule — containing the hydrophobic d5SICS:dNaM base pair (Fig. 1), thus creating the first organism to harbour an engineered and expanded genetic alphabet. This feat was far from simple: the authors first had to find a way of getting the bacterium to take up unnatural nucleotides, and then to work within the constraints of the billion-year-old habits of polymerases, the enzymes that synthesize polymeric nucleic acids.

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To solve the first problem, Malyshev and colleagues engineered an *E. coli* strain that expressed an algal nucleotide triphosphate transporter

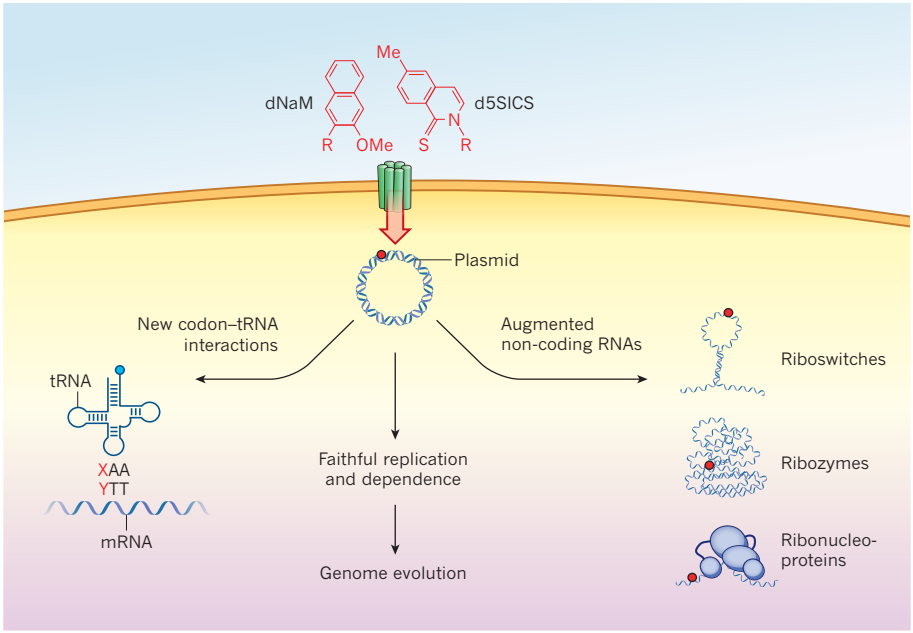

Figure 1 | Prospects for organisms that propagate unnatural DNA base pairs. Malyshev *et al.*[2] have generated a strain of the bacterium *Escherichia coli* that expresses an algal transporter protein (green), which imports the unnatural nucleotide triphosphates dNaM and d5SICS from the culture medium. This allows the bacteria to replicate a plasmid that incorporates the unnatural dNaM:d5SICS base pair (red dot). In turn, this might open up many further developments, including: organisms that can add new codons to the genetic code through customized codon–transfer-RNA interactions (XAA and YTT are the anticodon and codon of the tRNA and messenger RNA, respectively; X and Y are unnatural bases, A and T are natural bases); organisms that faithfully replicate and depend on unnatural base pairs, which might allow genome evolution; and non-coding RNAs (such as riboswitches, ribozymes and those in ribonucleoproteins) that have augmented functions. Me, methyl group; R represents the sugar and phosphate groups of the nucleotide triphosphates.

(NTT) protein, which allowed direct import of the nucleotides d5SICS and dNaM. To ensure efficient replication, the authors placed the unnatural base pair in a region of a plasmid predicted to be replicated solely by DNA polymerase I. Rather than being the workhorse of DNA replication, this enzyme fills in gaps in DNA molecules or connects 'Okazaki' DNA fragments, and has been shown[9] to replicate the d5SICS:dNaM pair efficiently *in vitro*.

After introducing a plasmid containing a single d5SICS:dNaM base pair into *E. coli* and supplementing the media with the two unnatural nucleotides, the researchers demonstrated that the unnatural base pair was retained in the plasmid after days in culture. They proved the presence of the unnatural base pair in recovered plasmids using a battery of techniques. Retention of the unnatural base pair after 15 hours of cell growth and plasmid replication was estimated to be at least 99.4% per doubling of the plasmid, an error rate no worse than that of some viral polymerases.

The next step will be to ensure long-term retention, which may require the engineering of a bacterium that depends on the unnatural base pair. It may be that the biological machinery used in Malyshev and colleagues' *E. coli* will allow the organism to readily adopt the unnatural bases as part of its own genetic alphabet. If so, this would open up a new vista in which human engineering can leap chasms previously unfathomable to evolution. This may seem fanciful, but wholescale reassignment of the genetic code produced through several billion years of evolution also seemed unlikely, and has nonetheless recently been achieved[10].

Once unnatural base pairs are not just tolerated by an organism, but also accepted and used, the next crucial step will be to demonstrate that they can be transcribed into RNA *in vivo*. From there, the opportunities multiply quickly (Fig. 1) — for example, unnatural nucleotide pairs might augment functional RNA elements, such as riboswitches and ribozymes. The incorporation of unnatural nucleotides into DNA promoter sequences or repressor binding sites (which initiate or subdue gene expression, respectively, by acting as binding sites for proteins), in conjunction with engineering of their partner proteins, might be used to formulate new and independent regulatory architectures.

Similar engineering feats could also provide unique functionality to RNA–protein complexes, for instance, by restricting the binding of the Cas9 enzyme (a widely used tool for generating double-strand breaks in DNA) to sites containing an unnatural base pair. But perhaps the ultimate application of such base pairs will be to add novel codons — triplets of nucleotides that encode which amino acids are incorporated into proteins — to the genetic code through customized codon–transfer-RNA interactions. This would greatly expand the number of available codons that can be assigned new translational functions, such as encoding non-standard amino acids, and would prevent synthetic biologists from having to recode the translational functions of existing codons[10] through painstaking genome engineering. In other words, an expanded genetic alphabet will help build an expanded translational alphabet.

But why stop at six letters in DNA? The NTT used by Malyshev *et al.* may be fairly promiscuous, importing both natural and unnatural nucleotides indiscriminately. Other groups have developed unnatural base pairs[7,8] that could be equally acceptable substrates for the transporter and for the cellular replication machinery. If the technique for introducing d5SICS:dNaM into *E. coli* works for other pairs, then the DNA code could be extended well beyond three base pairs. This raises fundamental questions about why life settled on only two in the first place, and whether semi-synthetic organisms with the capacity to store more information will have expanded capabilities (as we envisage above) or endure intolerable fitness costs (owing to inherently lower fidelity of DNA replication, RNA misfolding or translation-error catastrophes).

Attempts to expand the genetic alphabet bravely question the idea of the universal nature of DNA, and potentially draw criticism about the wisdom of tinkering with it. Such criticisms should be solidly countered by synthetic biologists at the outset. James Watson and Francis Crick's discovery of base pairing in DNA yielded a mechanism for genetics, but now genetics has inexorably yielded a mechanism for greater biological diversity, and thus potentially for building a better biological future. ■

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