study of universal patterns of evolution such as the distribution of evolutionary rates of orthologous genes, which is nearly the same in organisms from bacteria to mammals [20] or the equally universal anticorrelation between the rate of evolution and the expression level of a gene [21]. The existence of these universals suggests that simple theory of the kind used in statistical physics might explain some crucial aspects of evolution.

It is too early to tell whether or not these directions and others can be combined into a new evolutionary synthesis in the foreseeable future. I will venture one confident prediction, though. Those celebrating the 200th anniversary of the *Origin* will see a vastly different landscape of evolutionary biology.

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Research Focus

Reading between the LINEs to see into the past

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Transposable elements (TEs) are an important source of genome diversity and play a crucial role in genome evolution. A recent study by Zhao et al. describes novel patterns of TE diversification in the genome of the extinct mammoth *Mammuthus primigenius*. Analysis of *Mammuthus* has provided a unique genome landscape, a pivotal species for understanding TEs and genome evolution and hints at the diversity we verge on discovering by expanding our taxonomic sampling among genomes. Strategies based on this work might also revolutionize investigations of the interface between TE dynamics and genome diversity.

Discovering TEs in the mammoth genome

TEs (Box 1) have had a substantial impact on eukaryotic genomes throughout history, and are responsible either directly or indirectly for much of the genomic diversity we see today. Unsurprisingly, studies of TE impacting on human and non-human primate genomes are numerous and well developed. We know, for example, how the movement of TEs has influenced human disease [1], genome size [2–8] and the transcriptome [9–11]. But how well does our little corner of the genomic world reflect TE diversity and impact in a more general sense? The broader mammalian perspective is only now being investigated, and although we are starting to answer this question [12], many gaps in our knowledge remain.

Recently, Zhao et al. [13] applied next-generation sequencing (454) to address the question in a unique way – by investigating the TE amplification dynamics in the woolly mammoth (*Mammuthus primigenius*), a species that has been extinct for ~10 000 years. Using the massive amount of data available from the mammoth genome project, they determined likely TE content using an iterative

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Box 1. Transposable element structure

Transposable elements (Figure I) are repetitive DNA sequences that accumulate in genomes via multiple mechanisms. Class I elements (a), the retrotransposons, utilize a 'copy and paste' method referred to as retrotransposition. With these elements, the original DNA copy in the genome is first transcribed to mRNA. This transcript is often used as a template by reverse transcriptase (RT) to form a DNA molecule that is then inserted into a new location in the genome via a process known as target-primed reverse transcription [47]. There are several subgroups of class I elements including the LTR and non-LTR retrotransposons (including LINEs). Autonomous elements within these categories encode much of the enzymatic machinery required for mobilization. In the case of LINE/RTE, the principle class I element described by Zhao et al., one or two open-reading frames (ORFs) provide endonuclease (EN) and RT activity. The LTR element structure differs from LINEs in the identity and organization of ORFs. Often, they include a virus-like integrase (IN) coding sequence, and do not contain a tract of direct repeats (DR; e.g. the poly-A stretches at the end of Alu SINEs).

Class II elements (b), the DNA transposons and their derivatives, are common in many organisms from bacteria to humans. First discovered by Barbara McClintock in maize, DNA transposons differ from class I elements in that they often utilize a 'cut and paste' mechanism. In other words, the entire DNA segment of autonomous elements is excised from where it resides and reinserted into the genome at a different location. This is accomplished via an encoded transposase. Surrounding the transposase ORF are 5′ and 3′ untranslated sequences and terminal inverted repeats (TIRs), which are the recognition target of the transposase during mobilization. The Tigger1 transposon was mentioned in Zhao et al. as potentially recently active in the mammoth.

Non-autonomous elements exist within both classes. These typically short elements rely on an autonomous partner to provide the enzymatic machinery for their mobilization. Two SINEs, common non-autonomous partners of LINEs, discussed in detail by Zhao et al. were AfroSINE and AfroLA. These tRNA-derived SINEs are similar in structure but differ in their temporal distribution with AfroSINEs spreading throughout the genome much earlier than AfroLAs. Non-autonomous DNA transposons (various MERs) were also recovered but seem to be ancient and inactive. In all cases, the arrows represent target site duplications generated on insertion.

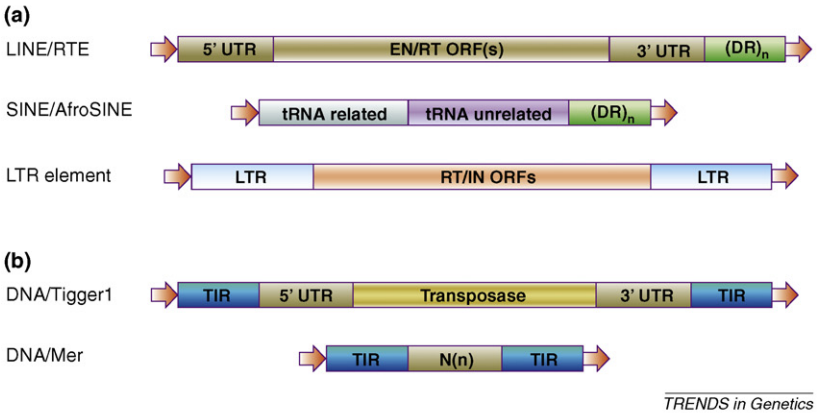

Figure I. The varied structural features of TEs.

process to identify relatives of known TE families. It was immediately clear that the sheer volume of TEs within the mammoth genome sets it apart from other mammals. The uniqueness of the mammoth genome can been seen in several TE-related areas, including genomic expansion, TE diversity and a likely case of horizontal transfer of a class I TE. Such observations hint at the tremendous potential for finding a vast array of TE-associated diversity in mammals as their genomes are explored. Zhao et al. also demonstrated the impressive potential of next-generation sequencing with regard to subgenomic analysis. The increased throughput provided by platforms such as the 454, Illumina and SOLiD systems has revolutionized numerous aspects of biological analysis from gene discovery to expression profiling and whole genome sequencing. In particular, the combination of high-throughput DNA sequencing and the repetitive nature of TEs make certain next-generation sequencing platforms ideal for rapid and inexpensive genome-wide TE analyses. Here, we suggest how researchers can use next-generation sequencing approaches to implement broad genome-wide surveys of TE content, and discuss the likely impact this will have on our understanding of the wider role of TEs. Such strategies could radically alter our ability to investigate and understand the complex interface between TE amplification dynamics and genome diversification.

TE content and genome dynamics in the mammoth

The mammoth genome contains a greater proportion of TEs than any mammal analyzed to date. This led Zhao et al. to highlight the potential connection between increased genome size (~50% larger than our own [14,15]) and the rapid expansions of particular TEs. Increased genome size has long been considered a potential consequence of TE expansion [16]. Many mammals seem to have accommodated massive TE-mediated genome expansions, whereas certain animals (e.g. birds, reptiles and some fish) have had a tendency to eliminate them [17–21]. For example, analysis of the recently sequenced genome of *Anolis carolinensis* revealed that although these lizards have several recently active lineages of long interspersed elements (LINEs), they have essentially reached equilibrium between TE insertion and removal over the past several million years [18]. Observations of the mammoth genome, along with many other comparisons between mammalian and non-mammalian taxa, suggest that we and our hair-bearing relatives share a unique ability to accommodate some TE expansions while repelling others. Several hypotheses have been advanced to

explain this observation including the utilization of DNA methylation as a control mechanism [22–24], decreased ectopic inter-element recombination [21,25] and increased permissiveness for some families to allow for inter-element competition and selection [21].

It is important to note, however, that the mammoth genome expansion was probably not the result of the common mammalian LINE L1, but instead seems to be the result of both L1 and a nearly parallel RTE (one of 11 well-defined lineages of the LINEs [26]) element expansion. Zhao et al. found that as much as 12% of the mammoth genome consists of RTEs, whereas the mammal with the next highest RTE proportion is the opossum at a mere 2.3% [27]. Thus, the mammoth is the first eutherian genome characterized to have accommodated multiple simultaneous LINE expansions. Most other TE types [short interspersed elements (SINEs), long terminal repeats (LTRs), LINEs (L1 and L2) and DNA transposons] in mammoth DNA were on par with or in fewer numbers than in other mammalian genomes. Significantly, RTE elements are absent in armadillo [13], cetartiodactyls, primates, carnivores and rodents [28], but are found in ruminants and at least two Afrotherian clades, namely tenrec and the modern elephant [29] (Figure 1). This distribution of RTEs lends support to the idea that each repeat is a unique genomic invasion by a lineage of LINEs with an unprecedented ability to spread via horizontal transfer [28,30,31].

Although not the main focus of the study, Zhao et al. also noted the relatively recent activity by Tigger1 (a class II element or DNA transposon) in the mammoth genome. With the exception of a single bat genome that has experienced multiple massive waves of recent DNA transposon activity [32–34], many mammals including *M. primigenius* have repelled DNA transposons rather successfully. However, this Tigger1 expansion adds to a growing number of studies suggesting that there are rare occasions in which mammalian genomes are impacted by single class II lineage expansions [35,36]. The increasing number of these isolated instances demonstrates that a general shutdown of mammalian class II TEs, as suggested by numerous studies [37,38], has been subverted by selected elements via multiple instances of horizontal transfer into some mammalian genomes.

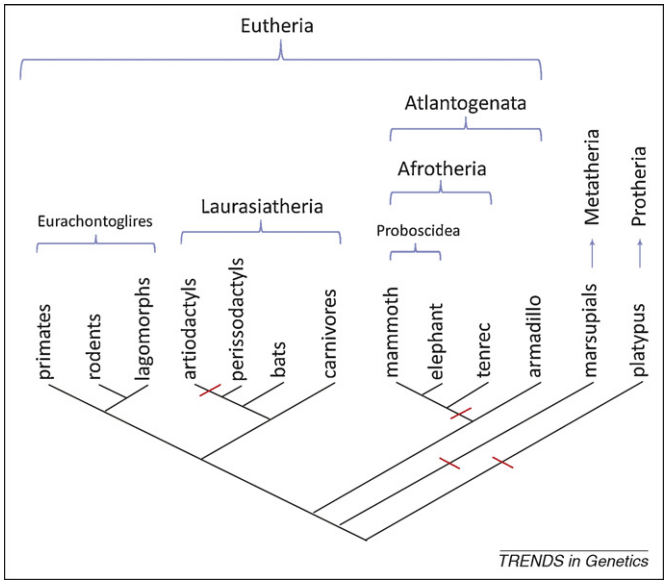

Figure 1. Phylogenetic relationships of selected mammalian clades with the hypothesized position of mammoth included. The phylogeny reveals the basal position of Afrotheria (and the mammoth) within Mammalia, specifically eutheria. With morphologies as diverse as that seen in the fossorial, golden mole to the slow swimming manatee, many studies have had difficulty deducing interordinal relationships within Afrotheria because of rapid evolution and cladogenesis. Studies incorporating TEs as phylogenetic markers [42,48] within Afrotheria have augmented large-scale DNA sequence studies [49] to better understand this rapid diversification. An understanding of the unique TE dynamics within this group might eventually enable a deeper understanding of the unique evolutionary pressures that allowed it to diversify so successfully. The red lines indicate the likely points of invasion of RTE elements based on their taxonomic distribution.

These observations suggest that our focus as researchers is to now determine the answers to several likely interrelated questions. What is the mechanism of these horizontal TE transfers, both class I and class II? What makes some genomes more susceptible to and/or tolerant of TEs and novel genomic invasions by TEs? Why are some TE families, such as RTE, better able to 'jump' between genomes than others? It is clear that the answers to these questions will not be found by the relatively limited sampling of genomes currently available to us. Instead, the extensive study of a wide variety of mammalian and non-mammalian genomes will be necessary to answer any one of these questions. Fortunately, the data presented by Zhao et al. provide a start to the process by expanding our knowledge of TE dynamics to the taxonomically important mammalian lineage Afrotheria.

The importance of Afrotheria

Most genomic sequencing and analyses tend to be focused on biomedical model species, such as *Rattus*, *Mus* and various non-human primates [38–41]. However, a thorough knowledge of mammalian TE dynamics is only possible when appropriate outgroups are also examined. One of the more important aspects of the *M. primigenius* analysis is that the authors chose to study a unique and important mammalian lineage that serves as a basal group within the class Mammalia. After the metatherian–eutherian divergence ~105 million years ago (mya) [42], it is estimated that the earliest diverging clade of extant mammals Atlantogenata arose quickly (~103 mya) [42], almost 20 million years before the next major divergence Laurasiatheria [43]. As a member of Atlantogenata, Afrotheria is one of the earliest diversifications of Atlantogenata (Figure 1). Consequently, Zhao et al.'s analysis of *M. primigenius* is an important contribution to the study of genome dynamics associated with the protherian, metatherian and eutherian diversifications.

Subgenomic targeting using next-generation sequencing technology

Of broader significance, Zhao et al. have successfully harnessed the power of next-generation sequencing to target a particular genome component. Indeed, the power of next-generation sequencing for understanding the evolutionary patterns of repetitive sequences and their impact on genome evolution has recently been shown in work on the pea (*Pisum sativa*) [44] and soybean (*Glycine max*) [45] genomes. The use of 454 sequencing technology seems particularly well suited to genome-wide TE analysis [44]. As seen from Zhao et al.'s analysis on the mammoth

genome, the relatively long average read-lengths of 454 technology (~250 bp for FLX chemistry and 400 bp or more for Titanium chemistry) enabled the identification of full insertions of smaller, nonautonomous elements, such as SINEs and MITEs (miniature inverted-repeat transposable elements). This task would have been difficult for technologies with a higher throughput but considerably shorter reads (e.g. Illumina and SOLiD) of the order of 75+ bp. It is now clear that in complex genomes (e.g. the plants examined by Macas et al. [44] and Swaminathan et al. [45], and now, the extinct *M. primigenius*) TE content can be accurately surveyed using the random genomic fragments targeted by 454 pyrosequencing.

What is the best way to use the sequencing tools available? For example, can we examine multiple taxa in a single 454 run? We suspect that this might be an efficient strategy. Using the older FLX chemistry, Macas et al. [44] obtained ~33 Mb of data from the 4.3 Gb pea genome (~0.77% of the genome), with the data consisting of 319 402 reads with an average length of 104 bp. Using this data, they identified what are likely to be all of the major TE families in the genome. The new Titanium 454 chemistry from Roche promises one million reads averaging 400 bp, or 0.4 Gb of data. Assuming an average mammalian genome size of 3.3 Gb (http://www.genomesize.com), a single Titanium run would provide a researcher with a random sample of 12% of a single genome, much more than is necessary for surveying mammalian TEs. It would, therefore, be more cost effective and efficient for a researcher to subdivide the run among 10 taxa (1.2% of the genome for each), thereby generating the data for a taxonomic survey of TE dynamics in a cluster of taxa rather than just one. Consequently, we would be making large-scale genome-wide comparisons and investigations of TE dynamics in a broad range of taxa the norm rather than the exception.

Concluding remarks

By providing a thorough analysis of TEs within the mammoth genome Zhao et al. have advanced the quest to understand TE dynamics in mammalian genomes. Although the mammoth has some typical mammalian genome qualities, such as a relative lack of recent DNA transposon activity, other characteristics, such as the increased activity of RTEs and larger overall size, make this genome unique, further highlighting its significance. The data also point to the potential variety of mammalian TE dynamics that might be just around the corner given our rather limited sampling to date.

Still, several questions remain. For example, how does TE diversity impact species diversity? In some cases, we see a correlation between the two, for instance consider the bat genus *Myotis* with its massive class II TE activity within the same historical period of its worldwide diversification into 100+ species [33]. In other cases, there is no obvious connection; the bat genus *Pteropus* is nearly as species-rich but seems to have experienced a shutdown of all TE activity [46]. Obviously, mere TE activity is not enough to ensure diversification. Under what ecological and genomic conditions would such activity contribute to adaptive radiations?

What is the mechanism through which the observed instances of horizontal transfer might occur? There must be a vector by which transferred elements are moved from genome to genome. The most obvious place to look would be among the blood-born parasites and the pathogens (viruses in particular) that they harbor. However, probing random parasite and pathogen genomes would be a rather inefficient methodology.

Depending on taxon selection, the goals of both studies could be easily addressed. For example, examinations of taxa sharing similar ecological niches but distinct taxonomic distributions and levels of species diversity might be a way to investigate whether TE expansions have affected species diversity. Alternatively, targeted 454 sequencing of parasites shared among organisms known to have participated in horizontal transfer events might yield results of interest to researchers attempting to identify the mechanisms of the horizontal transfer of elements.

Regardless, this study and others make it clear that we are entering a new phase in genomic research. By utilizing high-throughput genome sequencing and available computational tools efficiently there is little reason for us not to gather genome-scale data in an effort to investigate the interface among TE dynamics, genome change and species diversification.

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Research Focus

Sex determination: the power of *DMRT1*

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***DMRT1*, a mammalian gene related to sex-determining genes in flies and nematodes, is located in a region of the human genome that is important for sex development. This suggests that a common thread might run through the evolution of sex-determining mechanisms from worms to humans. New data show that *DMRT1* can cause sex reversal in chickens, adding support to this hypothesis.**

The great diversity of animal sex-determining mechanisms

An important principle in developmental biology is that gene regulatory pathways orchestrating the formation of important cells and tissues tend to be highly conserved through evolution, and so are often similar across diverse animal species. A classic example is found in eye development, where the same regulatory genes, *Pax6* and *Eya1*, are used from fruit flies to humans. This appears not to be the case with sex determination, where a variety of mechanisms is used throughout the animal kingdom to trigger the development of either a male or a female from a sexually ambiguous embryo (Figure 1). In mammals, most other vertebrates and invertebrates, this development is determined by chromosomes and, in some reptiles, by environmental factors such as temperature. Even where sex is determined chromosomally, two different systems can be used: an XX/XY system, in which embryos with two of the same sex chromosome (XX) are female and embryos

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