and results in killing of the target cells.

During amoebiasis, *E. histolytica* resides in the colon of an infected individual, where it depletes it of mucus, interacts with the exposed enterocyte cells lining the colon, dismantles the junctions between them and causes their death by lysis. The previous model[6] for the parasite's action was that it attaches itself to host cells and kills them, by an as-yet unclear mechanism involving the insertion of 'amoebapore' peptides into the cell membrane and subsequent lysis. It was also thought that the parasites engulf and ingest dying enterocytes by phagocytosis. But Ralston *et al.* instead show that the parasite ingests pieces of the host cell, and that this nibbling occurs in a repeated manner that ends up killing the cell. Once killing is achieved, the amoebae move on (Fig. 1).

This trogocytosis-like process is fundamentally different from amoebic phagocytosis, in which the parasite entirely ingests cells such as red blood cells (including dead cells). Although it is not known what determines the parasite's choice between phagocytosis and trogocytosis, some of the authors' findings point to mechanisms similar to those suggested in immune cells[7] that can perform trogocytosis in parallel with phagocytosis. For instance, both processes require an active actin-rich cytoskeleton and associated signalling pathways.

Interestingly, the authors' data show that parasites that have previously performed trogocytosis are 'primed' for higher ingestion activity and elicit more host-cell killing than parasites that have not, demonstrating that trogocytosis changes the parasites' behaviour. The authors have also produced images of trogocytosis in living tissue for the first time, and show that the process is required for the parasites to invade the tissue, leading to the pathogenic consequences for the host. They further show that the parasites can trogocytose all of the cell types tested, including enterocytes, lymphocytes, intestinal-tissue cells and red blood cells; because these cells have different surface constituents, it seems likely that a ubiquitous interaction occurs at the cell surfaces during trogocytosis.

It can thus be argued that the first step in amoebic trogocytosis — cell-to-cell attachment — is mediated by general components of the cell surface (for example, the glycocalyx, which is rich in glycoproteins and glycolipids), rather than by specific cell receptors such as the T-cell receptor (TCR), as is observed in immune-cell trogocytosis. The most relevant glycosylated (carbohydrate-containing) components on the parasite cell surface, in terms of abundance, are lipopeptidophosphoglycans and Gal/GalNAc lectin[6]. Glycosylated residues on these amoebic components might become attached to glycosylated components on the donor cells. The data obtained by Ralston *et al.* support this hypothesis, because Gal/GalNAc lectin is essential for the process.

Despite their respective cell specificity and ubiquity, there are interesting correlations between the TCR and Gal/GalNAc lectin in terms of signal-transduction characteristics: antigens bind to the TCR with relatively low affinity, similar to the predicted weak interactions between glycosylated residues and the Gal/GalNAc lectin; the activation of both intracellular signalling pathways involves the dynamic linking of the cell-surface molecules into microclusters[8,9] and requires a short cytoplasmic domain; and both pathways are associated with Src-kinase activity in the acceptor cell. However, these are only correlated features, because there is no evidence for structural homology between the TCR and Gal/GalNAc lectin.

It is also interesting to speculate on the significance of the intensification of amoebic trogocytosis in primed parasites. One possible explanation is that surface components of the host cell activate specific signalling pathways that enhance the parasite's affinity for extracellular carbohydrates. This idea is based on recent findings indicating that carbohydrate metabolism is involved in amoebic pathogenesis[10]. Are these signals necessary for killing donor cells by amoebic trogocytosis, but not for lymphocytic trogocytosis? Comprehensive comparisons of gene expression between primed and naive parasites (and in lymphocytes) might help to explain the roles of these activated signals. Further studies may also reveal whether amoebic trogocytosis and phagocytosis occur simultaneously, and what specific signals result from each process. But although such details remain to be determined, this new concept of amoebic trogocytosis is important for understanding not only amoebiasis, but also host–pathogen interactions in other systems and in immune-cell function and interactions. ■

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1. Hudrisier, D., Riond, J., Mazarguil, H., Gairin, J. E. & Joly, E. *J. Immunol.* **166,** 3645–3649 (2001).
2. Davis, D. M. *Nature Rev. Immunol.* **7,** 238–243 (2007).
3. Joly, E. & Hudrisier, D. *Nature Immunol.* **4,** 815 (2003).
4. Ralston, K. S. *et al. Nature* **508,** 526–530 (2014).
5. Aucher, A., Magdeleine, E., Joly, E. & Hudrisier, D. *Blood* **111,** 5621–5628 (2008).
6. Faust, D. M. & Guillen, N. *Microbes Infect.* **14,** 1428–1441 (2012).
7. Daubeuf, S. *et al. J. Immunol.* **84,** 1897–1908 (2010).
8. Chen, L. & Flies, D. B. *Nature Rev. Immunol.* **13,** 227–242 (2013).
9. Blazquez, S. *et al. Cell. Microbiol.* **10,** 1676–1686 (2008).
10. Thibeaux, R. *et al. PLoS Pathog.* **9,** e1003824 (2013).

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GENETICS

The vital Y chromosome

Comparisons of Y-chromosome sequences in various mammals reveal abundant gene loss early in the chromosome's evolution but remarkable gene stability across the Y chromosomes of extant species. SEE ARTICLES P.488 & P.494

ANDREW G. CLARK

The evolution of the mammalian X and Y sex-determining chromosomes from ancestral chromosomes is thought to have occurred through a rapid loss of genes from the Y chromosome. This idea of rapid degeneration[1] has been bolstered by observations made during the emergence of new Y chromosomes or Y chromosome segments in fruit flies[2]. In this issue, Bellott *et al.*[3] (page 494) and Cortez *et al.*[4] (page 488) present extensive accounts of gene evolution on the Y chromosome. They show that, although there was a period of rapid degeneration and gene loss during its early evolution, the genes that are conserved across the Y chromosomes of extant mammals (and the sex-determining W chromosomes of birds) have since been remarkably stable. The researchers' data also provide a detailed picture of the evolutionary forces acting on the sex chromosomes, and offer a plausible explanation for the functional coherence of Y-linked genes across these species.

The Y chromosome is notoriously challenging to study, in terms of both genetics and molecular biology. Despite the fact that male genomes were included in early whole-genome sequencing projects, the Y chromosome was largely ignored owing to the challenge of obtaining useful data from the chromosome, which is rich in repetitive and palindromic sequences. Bellott *et al.* adopted a previously described approach[5] — cloning regions of the DNA of interest into bacterial artificial chromosomes — to obtain and assemble DNA sequences from the Y chromosomes

50 Years Ago

'Extracorporeal perfusion of the isolated head of a dog' — Critical evaluation of cerebral metabolism and intracranial fluid distribution necessitates complete isolation of the brain's blood supply; however, brain viability must be demonstrated and maintained for such studies to be meaningful … In order to minimize handling of the brain substance, a factor which may disturb fluid distribution and cerebral metabolism, we have chosen to leave the brain within the skull during perfusion … Most cortical activity ceased when blood glucose was depleted … Even after the electrocortical activity ceased, corneal and lid reflexes remained intact and the oxygen and glucose consumption continued … From our experience, we believe that electrocortical activity is a sensitive index of brain viability, in that it is lost long before inactivation of corneal and lid reflexes or cessation of metabolism. In this preparation we have demonstrated that the dog brain maintains this activity for several hours after complete decapitation.
From *Nature* 25 April 1964

100 Years Ago

The second reading of a Bill to prohibit experiments on dogs was carried in the House of Commons on Friday last, April 17, by a majority of forty-two … It was stated on behalf of the Government that an amendment will be moved in Committee to abolish the proposed prohibition and to allow experiments only in cases where no other animal but a dog is available for the purpose … Before the second reading was taken, a memorial signed by more than three hundred eminent physicians, surgeons, and other scientific investigators, protesting against the measure, was addressed to the Home Secretary.
From *Nature* 23 April 1914

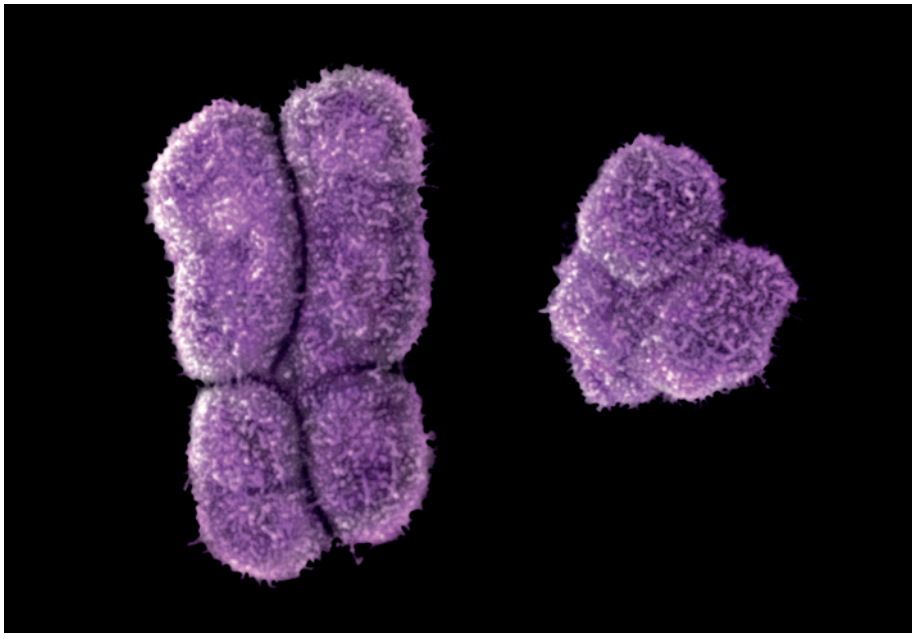
of four placental mammals (rat, mouse, bull and marmoset) and the marsupial opossum. They compared these with existing sequences for another three placentals (rhesus macaque, chimpanzee and human). Of the 184 genes that the authors infer to have been on the ancestral sex chromosomes some 300 million years ago, they find that only 3% survive on the Y chromosome of one or more of these mammals (Fig. 1).

Consistent with previous reports, this means that massive degeneration and gene loss did occur early in the history of the mammalian Y chromosome. However, once the genes had run this gauntlet, those that remained enjoyed remarkable stability on the Y chromosome. The authors also find that the 36 genes that are present on both the X and the Y chromosomes of all eight species they examined have maintained a stable presence for the past 25 million years. Ten genes were found to be shared across the Y chromosomes of the tammar wallaby, the Tasmanian devil and the opossum, indicating a stable Y-chromosome presence for the 78 million years of the marsupial lineage. These findings have important implications for our understanding of how natural selection acts to retain active functioning of specific subsets of genes on the Y chromosome.

Cortez *et al.* took a faster survey approach, in which they sought RNA molecules that are expressed in males but not females and then verified that the genes encoding these RNAs are found only in male genomic DNA. This allowed them to identify 134 genes transcribed from the Y chromosome across 10 mammals and to follow their evolutionary fates. By including the chicken (in which males have two Z chromosomes and females have one Z and one W chromosome) and the platypus (a monotreme that has a bizarre array of five X and five Y chromosomes), the authors were able to paint a broader picture of sex-chromosome evolution. Most noteworthy is their observation that the sex chromosomes of placental mammals, birds and monotremes had essentially independent origins, which means that patterns of gene loss and of specific retention of classes of genes on their Y (or W) chromosomes can be compared.

These data add depth and confidence to the model of evolutionary 'strata' on the sex chromosomes[6] that mark the time points at which X and Y sequences ceased recombining and subsequently diverged. Intriguingly, despite their independent origins, the authors find that the oldest strata in placental mammals, monotremes and birds are remarkably similar in age, estimated to have occurred 181 million, 175 million and 137 million years ago, respectively.

Another key aspect of genes on sex chromosomes is dosage sensitivity. Dosage-insensitive genes are those that function perfectly well when present as a single copy, and these are especially likely to become X- or Y-specific. By contrast, two copies of dosage-sensitive genes are required for normal health, and such genes are likely to be retained on both the X and the Y chromosome[7]. Genes involved in regulating gene transcription — such as those that encode transcription factors — commonly function inadequately in only a single dose, providing a hypothesis for why the Y chromosome has retained genes involved in transcription regulation.



POWER AND SYRED/SPL

Figure 1 | Small but stable. The human Y chromosome (right) is much smaller than the X chromosome (left), as a result of extensive degeneration early in Y-chromosome evolution. However, comparisons with other mammalian Y chromosomes by Bellott *et al.*[3] and Cortez *et al.*[4] show that there has been remarkable gene stability across Y chromosomes following this initial gene loss.

Because the Y chromosome is enriched with transcription-regulating genes, this means that it is far from being solely a male-determining switch that is flipped early in development. Instead, the Y chromosome has an impact on gene regulation across the genome in males, potentially influencing biological functions throughout life and in every tissue. It is fair to say that we are only beginning to understand the full extent of the differences in the molecular biology of males and females, and unanswered questions abound. For example, to what extent are male–female differences driven by specific interactions with Y-chromosomal factors?

In humans, the level of variation between individuals is considerably lower on the Y chromosome than on other chromosomes. However, Y-linked sequence changes can cause changes in gene expression across the genome, which could result in amplified differences among males. Despite the relative stability of the gene content on mature Y chromosomes, it is well known that DNA sequences evolve faster on the Y chromosome than on the X. Although this is generally perceived to be the result of the arrest of genetic recombination on the Y chromosome leading to reduced effectiveness of natural selection[8], it seems that the Y chromosome also has the potential to mediate remarkably rapid adaptive evolutionary change. ■

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1. Charlesworth, B. & Charlesworth, D. *Phil. Trans. R. Soc. Lond. B* **355,** 1563–1572 (2000).
2. Bachtrog, D. *Nature Rev. Genet.* **14,** 113–124 (2013).
3. Bellott, D. W. *et al. Nature* **508,** 494–499 (2014).
4. Cortez, D. *et al. Nature* **508,** 488–493 (2014).
5. Skaletsky, H. *et al. Nature* **423,** 825–837 (2003).
6. Lahn, B. T. & Page, D. C. *Science* **286,** 964–967 (1999).
7. Wilson Sayres, M. A. & Makova, K. D. *Mol. Biol. Evol.* **30,** 781–787 (2013).
8. Wilson Sayres, M. A., Lohmueller, K. E. & Nielsen, R. *PLoS Genet.* **10,** e1004064 (2014).

CLIMATE SCIENCE

Sea levels from ancient seashells

The isotopic composition of oxygen in sea water correlates with changes in global mean sea level. Microfossils carrying oxygen-isotope signals have been used to extend sea-level records as far back as 5 million years ago. SEE ARTICLE P.477

RALPH SCHNEIDER

On page 477 of this issue, Rohling *et al.*[1] present a convincing approach for calculating sea-level fluctuations over the past 5 million years (Myr). Their method depends on variations in the oxygen-isotope composition of shells produced by unicellular organisms called planktonic foraminifera (Fig. 1). In this way, the authors provide much-needed information that should help to predict future rates of sea-level rise in the event of complete or partial melting of the ice caps over Greenland and Antarctica in response to global warming.

The most recent instances of ice-sheet growth and melting that generated sea-level variation of several metres took longer than hundreds of years, making it impossible to determine their effects directly from historical records. To infer how growing and melting continental ice sheets affect sea level, at least the past 500,000 years must be considered. During this time, there were five periods of sea-level rise of up to 100 m or more, corresponding to the terminations of recent glacial periods (the intervals of time within the current, ongoing ice age that, in general, correspond to colder temperatures and glacier advances).

One could argue, however, that these terminations do not adequately describe what would happen during complete melting of the Greenland ice cap and parts of the West Antarctic Ice Sheet. Hence, it is much more appropriate to consider past conditions when Northern Hemisphere glaciation was still young — that is, when small, juvenile continental ice caps controlled sea-level fluctuations on timescales of tens to hundreds of thousands of years (the timescales associated with variations in Earth's orbit, which dominate the timing of glacial–interglacial cycles and sea-level changes). But how can this be done? The shells of foraminifera offer a potential solution. Made of calcium carbonate, they contain a record of the ambient isotopic composition of seawater oxygen during the organisms' lifetime.

Since the pioneering work[2] of the geologist Cesare Emiliani in the 1950s, it has commonly been accepted that periodic variations in the ratios of oxygen-18 to oxygen-16 ($^{18}O/^{16}O$) in foraminifera preserved in deep-sea sediments follow a global pattern characteristic of orbitally forced climate change during the Late Pleistocene epoch (about 700,000 to 11,700 years ago). Cool temperatures and great ice volumes both resulted in high $^{18}O/^{16}O$ ratios, whereas high temperatures and low ice volumes had the opposite effect. So, if the temperature effect can be disentangled from this isotopic record, then the remaining signal represents relative changes in continental ice volume. And if this signal can then be scaled to the amplitude of sea-level rise between glacial and interglacial periods (as has been done for the most recent postglacial period by correlating sea-level rises to the oxygen-isotope composition of cores taken from coral terraces[3]), then fluctuations in global sea level over time can be calculated, as long as ocean sediments provide continuous, undisturbed $^{18}O/^{16}O$ records.

Going back even further in time, a 5-Myr-long composite record[4] of $^{18}O/^{16}O$ ratios from foraminifera that lived in deep-sea sediments was until now considered the best chronicle of ice-sheet volume as Earth shifted from a hot, 'greenhouse' climate (about 55 Myr ago) to colder, 'icehouse' conditions (approximately 2.6 Myr ago). However, there are two problems with this record. First, the proportions of the temperature and ice-volume effects in it are unclear, because deep-ocean temperatures may have changed substantially over this long period of climate transition. Second, it is difficult to scale deep-sea oxygen-isotope variations at orbital timescales into robust estimates (including error margins) for the amplitude of sea-level rise and fall over the past 5 Myr.

Rohling *et al.* overcome these problems by converting $^{18}O/^{16}O$ ratios of fossilized planktonic foraminifera that proliferated in the surface waters of the eastern Mediterranean Sea directly into sea-level variations — an approach previously developed for a study[5] of the Red Sea, and which does not require temperature and ice-volume effects to be disentangled first. Their method depends on a hydraulic model of water exchange through the Strait of Gibraltar, which connects the North Atlantic Ocean and the Mediterranean Sea. This exchange mechanism not only controls the balance of evaporation and water renewal in the Mediterranean, but also strongly affects the seawater oxygen-isotope ratios recorded in planktonic foraminifera.

Assuming that there have been no major tectonic movements in the Strait of Gibraltar during the past 5 Myr that affect its depth and width, the oxygen-isotope signal from these foraminifera is simply a function of global sea-level variations relative to the modern hydraulic state of the Mediterranean Sea. The authors find that estimates of ancient sea levels relative