

A photograph of three men standing in a laboratory or office environment. The man on the left is wearing a light-colored, short-sleeved button-down shirt and dark jeans, smiling slightly. The man in the middle is wearing a white hoodie with black stripes on the sleeves and glasses, looking towards the right. The man on the right is wearing a dark, long-sleeved polo shirt with thin horizontal stripes and khaki pants, looking towards the middle man. The background shows laboratory equipment, including shelves with bottles and a large white container.

The spice of life

The reason that the vast majority of siblings aren't identical is because they inherit different combinations of their parents' genes. During the formation of every germ cell – an egg or a sperm – each parent's own genes are reshuffled. Each of their children therefore receives a unique combination of the genes of his or her four grandparents, rearranged and given an extra novel twist by any mutations that happened to be introduced during the production of the germ cells.

This reshuffling, called genetic recombination, creates the diversity within a species that is ultimately the secret to its strength. By exploiting that diversity, a species can adapt to a changing environment. However, this reshuffling also influences the inheritance of disease genes. Lars Steinmetz, Joint Head of the Gene Expression Unit at EMBL Heidelberg, and his group, together with the group of Wolfgang Huber at EMBL-EBI in Hinxton, UK, have now produced the most detailed map of genetic recombination to date.

In sexually reproducing species, cells undergo a process called meiosis, the duplication of their chromosomes followed by two nuclear divisions that gives rise to a haploid germ cell – that is, a cell with half the chromosome complement of its parent. In humans, for example, a sperm or an egg contains 23 unpaired chromosomes, compared with the 46 chromosomes found in pairs in other human cells. During fertilisation, egg and sperm fuse nuclei to produce a full set of chromosomes.

Recombination happens during meiosis, just before the first nuclear division, and it can take two forms: crossovers or

non-crossovers. In crossovers, chromosomes swap large segments or even whole arms. Non-crossovers are less dramatic, and involve copying small sections of DNA from one chromosome to another, with no reciprocal exchange. Both mechanisms help to shape the genome across subsequent generations, however, and both contribute to the breaking up of blocks of genes that would otherwise be inherited together.

Lars' and Wolfgang's groups created their recombination map for budding yeast, *Saccharomyces cerevisiae*. In Heidelberg, PhD student Eugenio Mancera mated two yeast strains and induced meiosis in them to produce around 200 haploid spores, or segregants. Lars chose the strains – one standard lab strain and one clinical strain, isolated from a patient – because their genomes, both of which had previously been sequenced, diverged sufficiently to provide enough points of comparison.

In all, there were 52,000 markers, or positions in the DNA sequence where the two genomes varied. These included insertions, deletions and, predominantly, single-nucleotide polymorphisms (SNPs) – places in the genome where different alleles or gene variants occur. Using arrays composed of DNA probes taken from the two parental strains, the researchers then 'interrogated' those markers in the spore genomes, to look for differences in behaviour. The arrays they chose were so-called tiling arrays, in which the probes either overlap or are very close together on the genome, providing a more unbiased analysis, and higher resolution, than traditional arrays in which the probes are further apart.



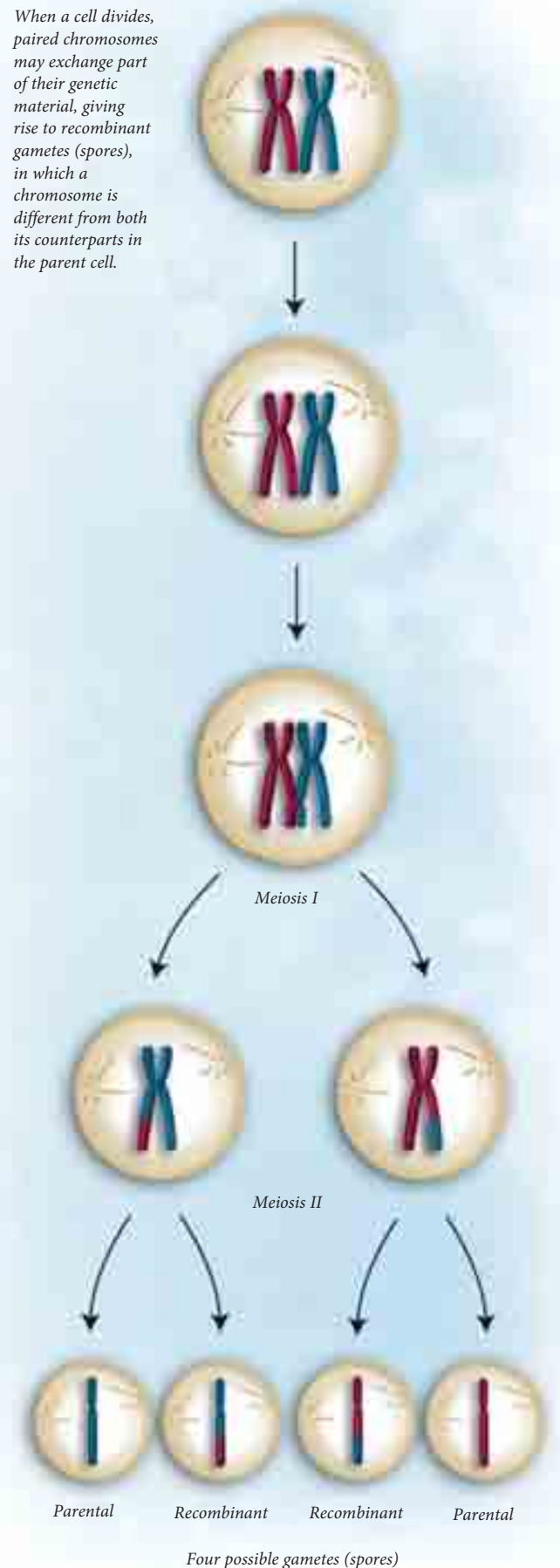
It was at this point that Wolfgang's group came in. A computer algorithm they had developed especially for the purpose, called ssGenotyping, used the data generated by the arrays to identify the genetic make-up, or genotype, of the spores at each locus the probes interrogated – that is, to find out which of the parents' genetic alternatives it had inherited at that place. To confirm the accuracy of their approach, they sequenced a handful of those loci, and compared the results with the genotyping calls returned by the arrays. "Once we had high-confidence genotype calls in hand, we could use these to infer the recombination events that each segregant had participated in," says Richard Bourgon from the Huber group.

Thanks to the combination of the Huber group's algorithm and the high-density coverage of the genome provided by the tiling arrays, the researchers produced a map with a resolution 20 times higher than that of any existing yeast map, and more than 360 times higher than that of a recent map of human recombination. "Traditionally, marker density has been low, so there have often been multiple recombination events that happened between two consecutive markers," Wolfgang explains. "With our technology we have multiple markers per recombination event. This means that we can detect these events unambiguously, look at their fine structure, and determine where in the genome non-crossovers have taken place."

The map reveals that about one per cent of the yeast genome undergoes sequence conversion during a single meiosis, or that more than 150 recombination events take place during a typical meiosis. For the first time it shows up non-crossovers across the whole genome – those events that have traditionally been so hard to see. The map also reveals that the recombination rate depends on the location in the genome, and that genomic 'hotspots' exist which favour either crossovers or non-crossovers. Previously, researchers had shown that crossovers can interfere with one another, making it unlikely that two would happen in close proximity. Now that the map has rendered non-crossovers visible, it has become clear for the first time that crossovers and non-crossovers can also interfere with each other.

"This knowledge is important to understand the mechanisms of how interference comes about," says Lars, adding that those mechanisms aren't yet understood, even for crossover-crossover interference. It is probably also important for understanding why there are differences in distribution of crossovers and non-crossovers, because interference may be the cause of those differences. Both types of recombination event are triggered in response to double-strand breaks (DSB) in DNA, which they repair. "If the

When a cell divides, paired chromosomes may exchange part of their genetic material, giving rise to recombinant gametes (spores), in which a chromosome is different from both its counterparts in the parent cell.





decision to follow one recombination pathway at one location of the genome affects the pathway decision at another location, then interference must happen before the pathways branch,” he says. “That could be as early as DSB formation.”

The existence of non-crossover hotspots also has implications for genetic mapping. Genetic linkage mapping is a technique used to determine the relative positions of genes on a chromosome and is used, for example, to link genes with an inherited trait. It relies on the principle that the closer together two genes are, the more likely they are to be inherited together. Or, to put it another way, the further apart they are, the more likely they are to be separated during meiosis by a recombination event.

To date, linkage maps have been built using observed crossovers, which are easier to spot than non-crossovers since

the exchange of DNA is reciprocal and hence, the effects larger. But non-crossovers can also break up gene blocks, through one-way transfer of genetic material. As Lars puts it, “Non-crossover hotspots create holes in linkage maps.” That means that conventional linkage maps may miss the essential detail when it comes to studying associations between genes and inherited traits. From now on, Lars and Wolfgang say, it is clear that if researchers want to capture all the genetic variation in a set of offspring, they will need to sequence the complete genomes of those offspring, in order to be able to identify the holes.

In the past, Lars’ group has studied correlations between meiotic recombination and phenotypic variation – that is, variation in the observable characteristics of an organism. Within that context, the two yeast strains he chose for the current study could provide an interesting comparison, since the clinical strain has some unusual features, including the fact that it grows at high temperatures and is capable of killing an immunocompromised mouse. “None of these traits exist in the standard lab strain,” says Lars.

He is confident that many of the same kinds of recombination behaviour that his and Wolfgang’s groups have uncovered in yeast will eventually be identified in the human genome, and that such information will prove useful in understanding how disease genes are passed from generation to generation. To date, around 2000 disease genes have been identified in humans which are inherited in simple, Mendelian fashion, and a few hundred that contribute in more complex ways to common diseases such as type II diabetes, hypertension and schizophrenia. Before scientists can accurately predict how common diseases are inherited, says Lars, two things must happen: “First we need to identify all disease genes and the intricate ways in which they interact, and second, we must have high-resolution maps of recombination in humans.”

Mancera E, Bourgon R, Brozzi A, Huber W, Steinmetz LM (2008) High-resolution mapping of meiotic crossovers and non-crossovers in yeast. *Nature* **454**: 479-85