Who would have thought that studying the genes responsible for a coat colour of the Belgian Blue would lead to the discovery of a new mechanism responsible for creating genetic variability? And yet this is what happened to Carole Charlier and her colleagues at the Unit of Animal Genomics. The results of their research were published in the journal *Nature* at the beginning of February. They are almost as much a part of our landscape as the furniture in our homes. You simply have to step outside of town to find them grazing in the fields. After more than 10,000 years of domestication, cows have become such common animals that although we humans regard them fondly, we often pass them by without a second thought. But while we might pay little attention to cattle, "cow" patterns, on the other hand, have aroused great interest. Indeed, the white coat with black spots is very fashionable, decorating bibs, dressing gowns, cushions, telephone covers, umbrellas, shoes, bins and other products.
and the mutations responsible for a **phenotype** by studying the whole **genome** thanks to statistical analysis programmes that allow you to locate these characteristics**, Carole Charlier points out. "For recessive diseases, this type of programme has been perfected and we would like to develop the same type of analyses for dominant characteristics", she continues. In the case of the the Belgian Blues, they have a dominant phenotype regarding a particular colouring of their coat. This is translated into pigmented sectors on the flanks and a white band along their spine known as "lineback". "*We thus hope to be able to identify new genes involved in bovine coat colour because these genes frequently have other more important effects on characteristics. For instance, in mice, some genes that play a role in the colour of these rodents’ coats are also associated with effects on fertility or obesity*", Carole Charlier elaborates.

**An inconclusive first round**

To find the gene containing the mutation responsible for the said phenotype, the researchers from the Unit of Animal Genomics, led by **Michel Georges**, went in search of a piece of ancestral chromosome - a segment of **chromosome** where the mutation originally occurred - common to all cows with this phenotype. "*We compared the genome of cattle with the "lineback" phenotype with that of the control cattle without this coat phenotype. And we found a region common to cows with the phenotype we are interested in on chromosome 29*", the scientist reveals. Unfortunately, this region was known for containing only one gene that was of no interest to this research. This gene isn't expressed in the **melanocytes**. "*We remained stuck at this stage for almost two years*", Carole Charlier remembers.

It is quite by chance that this project resurfaced: "**Keith Durkin, an Irish postdoctoral researcher who joined our laboratory, was working on another project aimed at establishing a catalogue of all the structural variants in the bovine genome**, the researcher continues. The structural variants, or structural **polymorphisms**, are all types of variations that can be observed within the genome of individuals of the same species such as deletions, duplications, translocations, inversions, and insertions of **DNA** fragments. "*To carry out his research, Keith used data produced by the laboratory over the past few years. This was genotyping data relating to the whole genome collected from the genome of 6000 to 7000 bovines from different breeds*", Carole Charlier points out. What the Irish researcher was actually looking for within this data were polymorphisms of a structural type referred to as copy-number variation, i.e., the deletion, duplication or multiplication of sections of DNA.

Out of the thousands of variants of this type that were revealed, Keith Durkin selected a number of them, either because of their large size, or because they contained genes that were potentially involved in interesting phenotypes. "*Among these structural variants, one of them contained the KIT gene known for generating a particular coat phenotype when it is mutated*", the scientist reveals. "And based on this, we also realised that the genome of a certain number of animals in our database contained this duplicated gene and that they all shared the "lineback"coat phenotype".
A duplication to complicate matters

Following this work, the researchers found themselves faced with two conflicting pieces of information: the location of the gene responsible for the "lineback" phenotype in Belgian Blues on chromosome 29, and the fact that the animals with this phenotype have a duplication of the KIT gene, the gene of chromosome 6, in common. Therefore, they had to reconcile these two pieces of information. "We thus put forward the hypothesis that the fragment of duplicated chromosome that contained this gene wasn't duplicated in the region of origin but had inserted itself elsewhere in the genome, i.e., in the place where we had located the gene responsible for the phenotype", Carole Charlier explains. This type of mechanism is known as duplicative translocation: instead of duplicating itself in tandem, i.e., alongside the original chromosome segment, the duplicated segment inserts itself into another chromosome.

In order to verify this hypothesis, Keith Durkin took the analyses further by using a technique that reveals precise chromosomal segments with the help of fluorescent probes (FISH technique). "He was thus able to show that the duplicated chromosome segment containing the KIT gene had indeed inserted itself into the chromosome 29 region that we had located during the first stage of this research", the scientist continues. But how did this fragment of duplicated chromosome come to be there? What processes had taken place between the excision from chromosome 6 and insertion into chromosome 29? The next step for the GIGA researchers was to answer these questions. "If the piece of chromosome 6 had inserted itself into chromosome 29 in a linear manner, we would have seen the following: a piece of chromosome 29 followed by a piece of the duplicated chromosome 6 contained between two insertion points, followed by the rest of chromosome 29",...
Carole Charlier describes. But this isn't exactly what the analyses using the whole genome resequencing technique showed. The result was somewhat more complex. "In short, the segment of chromosome 6 that had inserted itself into chromosome 29 had formed a circular intermediate between these two events", the researcher reveals. Thus, after having excised itself from chromosome 6, the duplicated segment circularised itself. And before inserting itself into chromosome 29, it then opened up again at another point, thus changing the initial order of parts composing it (see diagram).

From the Belgian Blue to the Brown Swiss and the Tibetan yak...

Some animals from the Brown Swiss breed also have the "lineback" phenotype. "We discovered that researchers from the University of Bern were working on the genetics of this breed's coat and we contacted them so that we could collaborate with them. We wanted to check whether this phenotype resulted from the same genetic mechanism in the Brown Swiss", Carole Charlier explains. Initially, this didn't seem to be the
The analyses they had already initiated had allowed them to locate the region of the genome responsible for this phenotype in chromosome 6, in a region close to the KIT gene", the scientist specifies. At first sight, these results were therefore incompatible with those found for the Belgian Blue.

But it would have taken a lot more to discourage the group of researchers from Liége! Once they had the Brown Swiss DNA samples in their hands, they carried out the same genetic analyses as those performed for the Belgian breed. "These analyses revealed that in the Brown Swiss, part of the duplicated piece of chromosome 6 inserted into chromosome 29 had excised and circularised itself, taking a fragment of chromosome 29, and had reinserted itself into chromosome 6 through a homologous recombination mechanism", Carole Charlier points out (see diagram).

Eager to continue now that they were getting somewhere, the researchers extended their analyses to a wide range of cattle breeds with the "lineback" phenotype. In both European and African breeds, and even the Tibetan yak, they found the same characteristic insertion points as in the Belgian Blue and Brown Swiss.
"Some breeds have the same allele as the Belgian Blue and others the same as that of the Brown Swiss, while others have both of these alleles", Carole Charlier continues. "Hence, an original unique event occurred - the one characteristic in the Belgian Blue - that was transmitted to all cattle breeds with the "lineback" phenotype. In some of these breeds, this was followed by the second event described in the Brown Swiss. In both cases, these events help to explain this coat phenotype for the majority of the breeds we studied".

**On the trail of similar events in other genomes**

The results of this research, published at the beginning of February in the journal *Nature* (1), took the Unit of Animal Genomics well beyond their initial goal. Indeed, while the analyses of the "lineback" cattle genome have allowed scientists to determine the chromosomal regions responsible for this phenotype, they have also led to the discovery of a brand new genetic mechanism.

As shown in this study, this duplicative translocation mechanism by means of circular intermediates lies behind the structural polymorphisms in the bovine genome. It isn't difficult to guess the rest of Carole Charlier's and her colleagues' reasoning: "This type of mechanism must exist within other genomes and may lead to the creation of particular phenotypes. Each time a chromosome segment translocates and inserts itself elsewhere, it is likely to modify genes in the insertion area where it can take a piece of gene with it. This type of modification could be the source of diseases or other defects in man for instance", the researcher explains. Finding the traces of this mechanism within other genomes is therefore the new mission the Liége team is determined to accomplish!