Aurochs genetics,

a cornerstone of European biodiversity

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Preface

In 2015 a report is written on Aurochs genetics, made possible by a grant from the Dutch Liberty Wildlife fund. This fund provided the Taurus foundation with a grant of EUR 20.000 to conduct genetic research on aurochs and its relation with nowadays so-called ‘primitive’ breeds. This is the summary of that report. This summary shortly describes the current state of affairs, what we do know early 2015 about the aurochs, about domestic cattle and the relationship of aurochs and the primitive breeds used in the Tauros Programme.

Nijmegen, December 2015.

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Summary

The Tauros Programme is an initiative of the Dutch Taurus foundation, since 2011 in close cooperation with Rewilding Europe and ARK Nature. The programme aims at rewilding cattle and wants to develop a credible replacement for the extinct aurochs. This replacement is called the Tauros. The Tauros will be based on the best available scientific knowledge, involving archeo-zoology, history, ecology, ethology and genetics.

In 2013 a funding of EUR 20.000 from the Dutch Liberty Wildlife Fund, provided by Rewilding Europe, was a new stimulus to the research. We already had been able to make a comparison between Aurochs and a list of 30 European primitive breeds based on the mitochondrial DNA (this is a small DNA-particle outside of the cell-nucleus). But with the funding from the Liberty Wildlife Fund we now – for the first time - were able to make the comparison between Aurochs and our 30 'primitive' breeds based on the DNA as it is found in the cell nucleus (autosomal[1] DNA). This type of DNA inside the cell-nucleus codes for the physical properties. This unique result could only be achieved because of the cooperation with a Dublin-based research consortium who very recently (2015) was able to sequence the complete genome of (one individual of) Aurochs.

A short summary of the results – at this moment – would be:

- We already knew that - based on the earlier analysis of mitochondrial DNA (mtDNA) which specifically inherits from mother to child - certain so-called Podolian breeds (breeds from Italy and the Balkan countries) show a close relationship with Aurochs. These results are based on the analyses of a greater number of aurochs individuals (since it's far easier to reconstruct these small fragments of DNA, than to reconstruct the complete genome);
- The information we now got from the comparison of Aurochs with our 30 primitive breeds based on the autosomal DNA, shows that Iberian breeds in general show a closer relationship to Aurochs. Since the information based on nuclear DNA tells us far more about inheritance, at this moment we consider the analyses of the autosomal DNA as the most valuable;
- 'Ranking the stars': One analyses (NEI genetic distance) calculated the distance between the analysed breeds in comparison to the one analysed Aurochs. It was found that four of the breeds that do play a major role in the Tauros breeding programme (three Iberian and one Podolian breed) are in the top 10 and six of our breeds are in the top 15.

These results lead to the following conclusions and recommendations for the breeding programme and follow-up research:

Conclusions

- The leading practise in the breeding programme so far – combining Iberian and podolian breeds/individuals with Aurochs-like physical properties – is being confirmed (by the combined results of autosomal and mtDNA and the NEI-analyses). However one should take in consideration that at this time only one specimen of Aurochs has been fully sequenced (autosomal DNA), so new additional information

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[1] Genetic jargon is marked orange when first used. A short explanation can be found in the glossary.

could lead to adjustments of this conclusion. It is therefore that the breeding programme still uses a rather wide variety of crossings (with the selected breeds);

- In the ‘top10’ a number of breeds is apparent that are not (yet) used in the breeding Programme. The reason for this was that on fenotypical features (the way the animals look) better breeds are available. However in future studies we need to look better at some of these breeds (such as the highranking Busha (not used in the programme because of its limited size)
- As the Podolica was ranked low, we should reconsider the use of this breed, which already was used in a very limited number.

Recommendations are:

Future research needs to dive deeper into the genetic features of Aurochs to be able to identify specific genes for domestication (and thus getting more ‘grip’ on genes that could be crucial for the opposite direction: the rewilding of the species).

More fully sequenced specimens of Aurochs that cover a substantial part of the European continent are needed, to be able to get an idea of the genetic diversity within Aurochs.

The research needs to result in practical guidelines – on the short run – as the base for future selection.

1 Introduction

The Tauros Programme is an initiative of the Dutch Taurus foundation, since 2011 in cooperation with Rewilding Europe and ARK Nature. The programme aims at rewilding cattle and wants to develop a credible replacement for the extinct aurochs. This replacement is called the Tauros. In the near and more distant future herds of Tauros will live as wild cattle and will contribute to the sustainable conservation of Europe's, open landscapes and biodiversity. At this moment, breeding groups of Tauros are already grazing areas in the Netherlands, Spain, Portugal, Croatia, Czech Republic and Romania.

The Tauros will be based on the best available scientific knowledge, involving archeo-zoology, history, ecology, ethology and genetics. During millions of years aurochs and its predecessors became genetically adapted to their environment. Therefore, the aurochs genes will tell us how to breed an effective replacement on the basis of the genetic variation of the primitive domestic cattle used for breeding the aurochs.

In 2013 a funding of EUR 20.000 from the Dutch Liberty Wildlife Fund, provided through Rewilding Europe, was a new stimulus to the research. This allowed a comparison of the autosomal[2] DNA's from 30 European primitive breeds with the DNA of the aurochs. In addition other breeds – such as the Croatian Boskarin and Maltese Cattle – were added to the database. The budget of the Liberty Wildlife Fund was also used for analysing the offspring of the Tauros crossbreds, a vital part of the total research, since without funding of this part of the research it would be impossible to judge whether or not the breeding is in the right direction. The funding of the Dutch Liberty Wildlife Fund was also used to write this report and by doing so providing broader access to the Tauros research.

On-going genetic research

In different parts of the world research groups are working on the genetic structure of aurochs. This started with the sequencing of mitochondrial DNA (mtDNA), which indicates the maternal lineage (i.e., it is transmitted from mother to calve) and is because of its abundance in a cell relatively easy to analyse. One milestone has been reached recently: the research group of David MacHugh (University College of Dublin) has been able to sequence the complete genome of a British aurochs from 6700 BP These results have been published recently[3]. So gradually the contours of aurochs genes are emerging. However, taken into account that once there must have been millions of these animals roaming for several hundred thousands years around Europe, Asia and Northern Africa, there must have been a substantial genetic diversity within the populations. With the genome of only one individual being available at this moment, it must be clear that we're still missing a lot of information.

The aurochs genomic sequence can be interpreted on the basis of our growing understanding of the role of genes and gene variants of cattle in determining

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[2] Genetic jargon is marked orange when first used. A short explanation can be found in the glossary.

[3] Park et al. Genome Biology (2015) 16:234 DOI 10.1186/s13059-015-0790-2

phenotypes as size, appearance (e.g., coat colour, shape of horns in cattle) and adaptation.

Survey of the Tauros Research Programme

In the Tauros Progamme a number of things has been tried to delve deeper into Aurochs genetics. In 2009/2010 parts of aurochs skeletons from museums all over Europe were collected, together with genetic material of around 30 different European cattle breeds with ‘primitive’ features. DNA was extracted and the genetic variation was measured at 770.000 sites (SNPs, see below) in the genome. This yielded most valuable new information on the genetic structure of ca. 30 primitive European breeds. We found that the mtDNA from aurochs and taurine cattle are different, and we could construct a first genealogical tree, sketching the genetic relation between these breeds and aurochs. There were disappointments too. DNA extracted from aurochs bones proved to be unsuitable for the genetic analysis, precluding for the moment a direct comparison of the aurochs and cattle autosomal DNA. This early part of the research was funded and carried out by Wageningen University, as part of their broader interest in the genetic structure of productive cattle-breeds.

In 2012 another attempt to sequence Aurochs genetic material was made. Part of the skin of a medieval specimen of a Polish aurochs was analysed by the research group of Beth Shapiro of the University of California, one of the foremost researchers in ancient DNA. However this lead to no result: the DNA proved to be too deteriorated. Fortunately, in 2012-2013, David MacHugh agreed to give Wageningen University access to the – at that moment still unpublished - sequence of the aurochs genomes, creating new opportunities.

In addition to the grant of the Liberty Wildlife Fund in 2013, in 2014 the Taurus foundation and Wageningen University were granted a European research budget of EUR 240.000 as part of the EGS-ABG programme. So in the autumn of 2014, a PhD student has started on a four-year programme at Wageningen University in cooperation with the University of Uppsala. The first results are being published early 2015.

A next contribution to our data set will be the DNA sequence of six to ten complete genomes of the important parent-animals of the breeding of the Tauros Program. This will be funded by grant of EUR 10.000 provided by the Dutch Province of Gelderland as part of their ‘Foodvalley’-programme and an additional EUR 10.000 provided by RE as part of the budget for the transport of Sayaguesa animals from Spain to Croatia.

The coming years the knowledge on the aurochs and on primitive cattle will be built up further. Additional specimens of aurochs will be analysed by the University College of Dublin and Dublin University - and our understanding of the history of domestication of cattle is growing (part of a research project of the University of Lyon).

Because important genetic information is still missing, we modified our initial choices. Instead of focussing on just two or three breeds to start with, we chose for a wider variety of breeds. This gives flexibility, for instance if on-going and future genetic research leads to new insights. A direct and most useful application of the genetic analysis carried out with the funding of Liberty Wildlife Fund is that we will be able to determine accurately the breed composition of the Tauros offspring and we are able to compare the offspring with Aurochs.

2 Aurochs: a short description

Archaeology, archaeozoology, written historical sources, cave paintings, knowledge about cattle genetics, cattle breeding and cattle behaviour have all contributed to our understanding of the aurochs in the last decades. We know what the animal looked like, we know its ecology,

The looks

The size of the aurochs bulls varied between 155 cm and 180 cm, depending on the local circumstances. The size of cows was between 135 cm and 155 cm. Bulls were significantly larger than cows. The body shape of an aurochs was different from many developed cattle breeds today. It had an athletic body shape and long legs, built for long-distance walking. It was a swift and very agile animal. The skull, carrying the large horns, was elongated and big compared to the rest of the body. Bull's horns could reach up to 107 cm in length and between 10 and 18 cm in diameter at their base. The cows had horns probably reaching up to 70 cm in length. The bull's horns were larger, with the curvature more strongly pronounced in cows. Both sexes had a distinct light-coloured, often white, muzzle and a distinct eelstripe (especially with bulls).

Range, habitat, ecology and behaviour

At the end of the last glacial period (12,000 BP) aurochs was endemic over most of Asia, Europe, North Africa and the at the time green Sahara (figure 2.1, from Felius et.al., 2014)

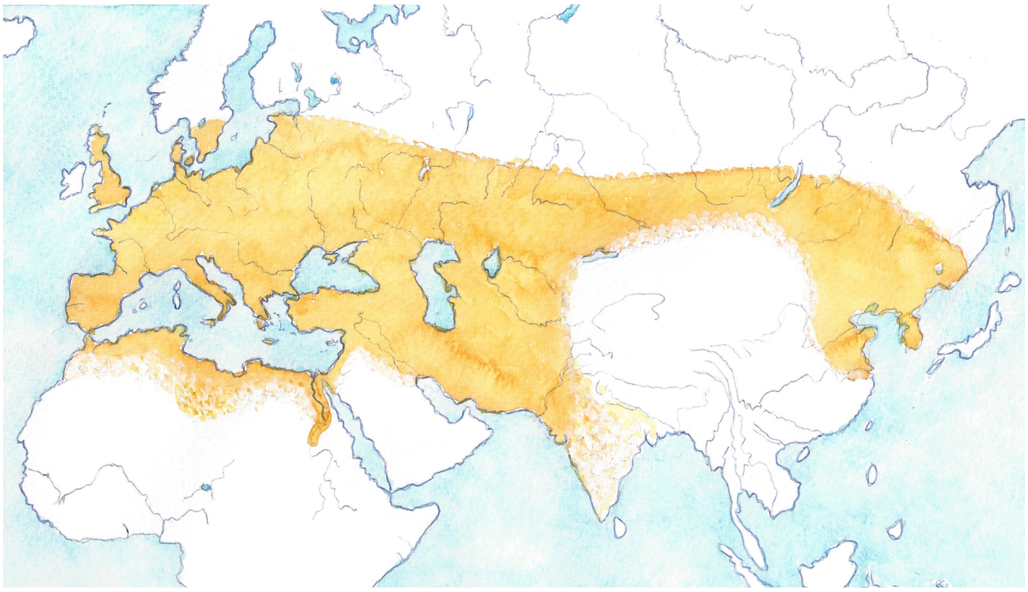

Figure 2.1: The presumed maximum area where Aurochs historically roamed around (map by Marleen Felius, Felius et.al., 2014)

Aurochs originated in India some 2 million years ago. During the Pleistocene epoch (2,5 million to 12,000 years ago) the species spread across other parts of Asia, also into northern Africa and Europe. The earliest remains of aurochs found in Germany date from about 400,000 years ago, but it is estimated the animal reached Europe long before that, probably around 700,000 years ago.

Aurochs habitat: forest dweller or king of the savannah?

Until recently, the prevailing theory was that aurochs mainly lived in closed canopy forests and forested riverine habitats or swamps. This view has been adjusted by ecological evidence and the studies on aurochs teeth, and skeletons, such as the recent study of Bakker et.al., 2015[4]. Although there's still some scientific debate, the viewpoint of Rewilding Europe and Taurus foundation is that Aurochs was a grass-eating plain animal. During the 'end-game' the animal was driven to a suboptimal habitat, the more or less closed forests, because men occupied the fertile plains. By their grazing activities aurochs opened up the vegetation and created biodiverse mosaics of vegetation.

Aurochs behaviour

Cattle don't run away from threats, but group together and defend themselves if needed. Therefore, cattle are not evenly distributed over the landscape, but form family groups and sometimes roam around in larger groups. This results in a temporary and locally high grazing pressure, depending on the seasonal food availability. The larger the area, the greater their effect is on differentiating the landscape.

Figure 2.1: Preferred habitat of aurochs

Under natural conditions, aurochs probably withdrew in summer to the cool mountains, where grass was more available than in the hot lowlands, while snow and cold forced them down again during the winter.

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[4] www.pnas.org/cgi/doi/10.1073/pnas.1502545112

Aurochs genetics

A lot of information is available on the genetics of cattle and the variability within and across breeds. The complete genome of a Hereford cow is available since 2009[5], On the genome about 22,000 genes coding for proteins have been identified. The role of many genes are now being discovered, with an emphasis on the economically important genes for milking gift, meat quality e.g.

The first studies of ancient Aurochs DNA focused on mtDNA. In 2010 the complete sequence of Aurochs mtDNA was described. Based on a recent mtDNA study of our modern cattle breeds it has been estimated around 80 females from the Fertile Crescent stand at the basis of all our nowadays cattle. Recently the complete genome of one British Aurochs has been analysed published (Park et.al., 2015).

In 2010 for the first time the complete mtDNA sequence of an aurochs (was established (Edwards et al 2010). This sample belonged to the aurochs haplogroup P (derived from 'primigenius'). In Italian Aurochs another haplogroup 'T' was found. Figure 2.1 shows the tree of the common cattle T mtDNA (here indicated as BRS), the aurochs P mtDNA and the sporadic Q and R mtDNA.

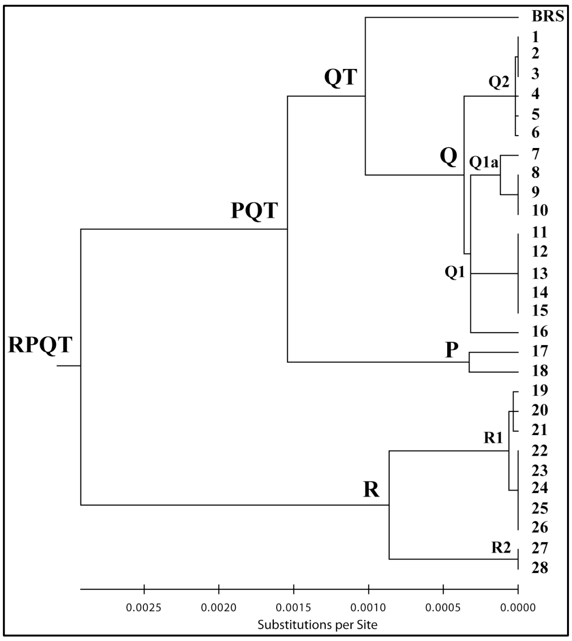

Figure 2.3: A tree of Bovine haplogroups of mtDNA[6]

Ancient DNA analyses

Among other methods, such as SNP genotyping and whole genome analyses, the current study uses ancient DNA-analyses as a source of information. Box 1 gives an overview of how this is done.

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[5] Elsik, C.G. *et al*, (Bovine Genome Sequencing and Analysis Consortium) (2009). "The genome sequence of taurine cattle: a window to ruminant biology and evolution". *Science* **324** (5926): 522–528. doi:10.1126/science.1169588. PMC 2943200. PMID 19390049.

[6] source: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0015760

Box 1 Ancient DNA (from Magee et.al., 2014)

The study of ancient DNA has the potential to answer questions about the past directly, thus providing a temporal aspect to modern genetic studies. Ancient studies have shown that DNA can survive intact for more than 500,000 years, enabling many evolutionary questions to be addressed over this time period, including biogeography, phylogeny, taxonomy and selective processes at the population level, as well as domestication origins. Ancient DNA can be extracted from a variety of animal remains, ranging in condition and age, the most popular of which are bones, teeth, and hair. The cytoplasmic location and high copy number of mitochondrial chromosomes within each eukaryotic cell has made mtDNA the primary genetic marker for ancient DNA studies. However, recent advances in genomic technologies have seen an increase in the number of ancient DNA studies that involve partial or whole nuclear genome sequences from archaeological samples (Shapiro and Hofreiter, 2014). Analyzing ancient DNA is a very specialized technology and requires dedicated facilities to prevent contamination with modern DNA.

3 The domestication of cattle

One of the main goals of the Tauros Programme is the de-domestication of cattle. Therefore it is important to summarize what we know on the domestication itself.

Early domestication: the 'covenant of the wild'

Until recently much of our knowledge on domestication was based on archaeological excavations, osteological analyses and radiocarbon dating. Nowadays our knowledge of early domestication of cattle has made big leaps because of the use of modern genetics.

We think that after a long period of co-evolution of man, aurochs (and wolf) (co-)domestication was the next step in this process. Domestication led to selective advantages for both partners: animals were provided with food and shelter humans with milk, meat and work supply. Domestication has been described as a "covenant of the wild" (Budiansky, 1994 in Magee et. al., 2014). Cattle were probably one of the more difficult species for domestication because of their size, but more easy because of their tendency to hold ground instead of fleeing (such as horses). They were amongst the first wild grazers to be domesticated.

10,000 BP: the start of it all

Around 10,750-10,250 years BP the domestication of Taurine cattle started in the Fertile Crescent (Mesopotamia). Molecular research has indicated that the number of aurochs first domesticated in the Middle East was very small, possibly only 80 individuals. Along the domestication routes wild-matings with wild aurochs bulls have taken place in Africa and Europe. The first selection on cattle-traits was directed on manageable size and docility. As a result of domestication, cattle began to decrease in size throughout the millennia that followed. Cattle were multipurpose and bred for meat, milk supply and working power. Bulls were castrated.

Routes of domestication

Domestication proved to be a very successful innovation. The first farmers soon needed new areas and took their animals along with them. This is where the myth of the founding of Europe - Zeus disguised as a bull with Europe on his back swimming from the Libanese coast to Crete - meets reality. Around 10,000 BP and from 8,500 BP, starting in the Fertile Crescent, a westward expansion of agricultural societies brought the first domestic taurine cattle to central Anatolia into Europe[7]. Another route was along the North-African Mediterranean coast and then along the West-African Atlantic coast. The typical African T1 mtDNA haplotype is also found in Iberian cattle, an indication of immigration of African cattle in Europe (Spain, Italy, Malta) across the sea straits. The migration further into Europe followed probably two routes: via the Mediterranean coasts and along the Danube river. Approximately 7,500 BP domestic cattle reached Central Europe via the Danubian route. Northern Europe followed ca. 1,000 years later.

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[7] Main source Felius et al 2014

During further domestication in Europe introgression of aurochs (bulls) may very well have taken place (and in fact has taken place as is proven in Park et.al., 2015). Groups of cattle were herded in remote areas and were not protected by barbed wire.

According to Decker et al (2014), indicine (from Bos Indicus) influence is found in three Podolian breeds (Chianina, Romagnola and Marchigiana), which probably descend from Hungarian steppe bulls imported during the 14th to the 18th century. Lenstra et.al. (2014) finds indicine influence in all Podolian breeds.

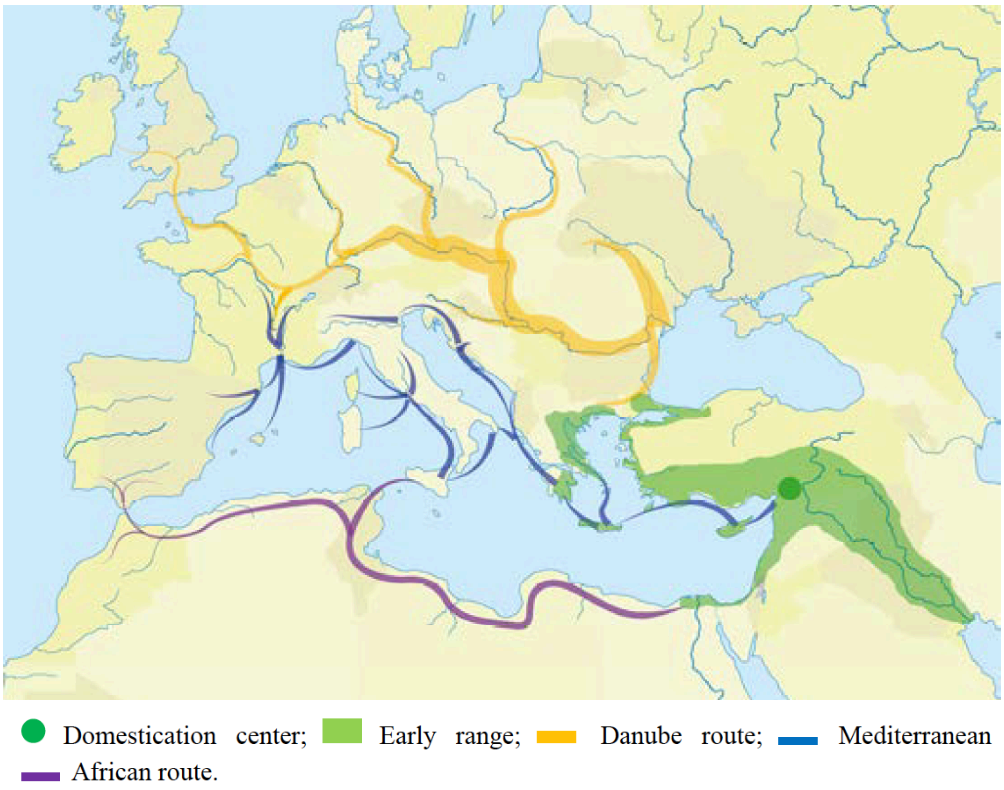

Figure 3.1: Domestication routes into Africa and Europe (from Felius et.al., 2014)

Bovine breeds

The migration of cattle to all inhabited continents, local adaptation and artificial selection has resulted into varieties of cattle breeds we observe today. Since the last 100-200 years, many cattle breeds have been intentionally modified to improve and produce the traits beneficial for human, resulting into high output cattle breeds specialized for specific purposes The profitable specialized cattle breeds have replaced several local and often extremely well-adapted traditional breeds. As a result, very many cattle breeds are at risk, most of them being the primitive cattle breeds. The mechanization of agriculture has undermined the maintenance of authentic draught cattle with primitive gene variants, leading to a further drift from the well-adapted natural grazer, the aurochs. A major concern is the crossbreeding of locally adapted bulls with highly productive breeds such as the Limousin.

Classification of European breeds

Decker et al., (2014) gives a classification of five major groups of breeds and within each group several clusters.

1. **North-European cattle**, comprising eight breed clusters.
2. **Central European cattle**, with many dual purpose (beef-work or dairy-work) and triple-purpose breeds, comprising three five breed clusters:
3. **Iberian cattle**, with a large variety of coat colours and horn morphology and mainly used as beef, work and fighting cattle;
4. **Grey Podolian cattle** ,the mostly long-horned and, primarily developed as beef and work animals;
5. **Balkan and Anatolian breed,** a group of genetically diverse breeds still representing the undeveloped taurine cattle.

The longlist of breeds for the Tauros Programme

In the Tauros Programme a first selection was made from the hundreds of breeds still preserved in Europe, the longlist. This selection was performed by the scientific committee of the Tauros Programme in 2009 and based upon specific features, history, purpose and management-system. Based on these criteria 38 cattle-breeds were selected and also used in the genetic analyses, including the breeds ((such as the Boskarin and Maltese Cattle) that were added later to this list. Annex 1 gives the total list.

Based on more detailed knowledge of the breeds a sub-selection of this longlist was used in the initial stage of the Tauros Programme, the shortlist. These breeds and the reasons for their initial selection for the Tauros Programme are listed in Annex 3. A breed as the Italian Chianina for example has been left out since the Podolian Maremmana primitiva with larger horns, less meat typical, better coloration and a more feral way of living is the better alternative.

4 Results

Research questions

In the end the Tauros Programme needs answers on the following questions:

- What was the genetic constitution of and variation within aurochs?
- Which genes in cattle have been changed in response to domestication?
- Which genes encode the essential aurochs-like features?
- What is the genetic constitution of the (primitive) cattle-breeds being used and how do they relate to each other and to the aurochs?
- How can we use this genetic information in the actual Tauros breeding programme?
- Does the Tauros Programme genetically move in the right direction: does the offspring approach the genetically aurochs?
- Will the resulting Tauros populations have enough genetic variation?

To provide these answers (at least the beginning of the answers) Wageningen University carries out the genetic research, in close cooperation with other scientific institutes. Annex 4 gives an overview of the different research-steps taken so far. The amount of research has been limited by the available budget. In the period 2010-2015 chronologically the following steps have been taken as part of the genetic research specifically oriented toward the Tauros Programme. The research specifically funded by the Liberty Wildlife fund was started in 2013 and concluded in 2015:

- Analyses of 100 crossbreds from the Tauros programme (inheritance check);
- 770 k SNP analyses of additional breeds (24 individuals);

This research is most relevant to the Tauros Programme, since without the inheritance check and the further analysis of the offspring generations, we will not be able to check if the programme moves in the right direction.

Methods followed

A number of different methods of analysis have been carried out: in general quite specialist genetic tools and methods, hardly understandable for non-geneticists. Therefore the methods are not described in the summary.

Results

The results of the different analyses are represented in three different ways. In Maulik et al (2015)[8] aditional analyses have been carried out and its results presented, however that does not change the general conclusions.

1. Calculation of the Nei genetic distance;
2. Population structure analysis by Model based clustering;
3. Parentage check.

NEI Genetic distance: Ranking the stars

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[8] Maulik et.al., 2015. Genetic origin, admixture and population history of primitive European cattle and Aurochs

Nei Genetic distance estimates the difference between breeds, a kind of ‘ranking the stars’. Nei Genetic distance shows how close different breeds are to the (one British) aurochs. Table 4.1 gives the results.

TABLE 4.1: GENETIC DISTANCE TO AUROCHS

Ranking	NEI Distance	Code	Breed	Country
	0	***AU***	***Aurochs***	***Britain***
1	0,1245	**PA**	**Pajuna**	**Spain**
2	0,1249	BU	Busha	Balkan region
3	0,1307	CA	Cardena	Spain
4	0,1312	**SA**	**Sayaguesa**	**Spain**
5	0,1365	FL	Fleckvieh	Switzerland
6	0,1374	BN	Berrenda en negro	Spain
7	0,1377	**LM**	**Limia**	**Spain**
8	0,1385	BC	Berrenda en Colorado	Spain
9	0,1386	LI	Lidia	Spain
10	**0,1390**	**MA**	**Maremmana**	**Italy**
11	0,1391	AR	Arouquesa	Portugal
12	0,1412	**ME**	**Maronesa**	**Portugal**
13	0,1443	BS	Brown swiss	Switzerland
14	0,1457	**HL**	**Highlander**	**Scotland**
15	0,1465	JE	Jersey	Jersey Island
16	0,1465	KC	Kerry cattle	Ireland
17	0,1481	GA	Galloway	Britain
18	0,1486	HE	Heck	-
19	0,1515	CH	Chianina	Italy
20	0,1520	RO	Romenian grey	Romania
21	0,1533	WP	White park	Britain
22	0,1536	**BK**	**Boskarin**	**Croatia**
23	0,1548	MN	Marondesa	Spain
24	0,1553	CC	Cachea	Portugal
25	0,1563	AL	Alentejana	Portugal
26	0,1582	**TU**	**Tudanca**	**Spain**
27	0,1587	DB	Dutch belted	The Netherlands
28	0,1600	MT	Maltese	Malta
29	0,1628	EL	English Longhorn	Britain
30	0,1638	MI	Mirandesa	Portugal
31	0,1923	AN	Angler	Germany
32	0,1925	CL	Caldela	Portugal
34	0,1978	**PO**	**Podolica**	**Italy**
35	0,2239	NE	Nelore	India

Table 4.1 shows that from the 34 primitive breeds analysed so far the four most frequently used breeds (marked) in the Tauros Programme are in the top 10. This is a remarkable result, since before 2015 the choice of breeds was made on the basis of the phenotype and the history of a breed. Especially the nr 1 ranking of the Pajuna breed is encouraging. With regard to the other breeds in the top ten: there were sound phenotypical reasons not wanting to use them in the initial phase, presented here below. However this does not mean that those breeds will not be used at all, because

apparently they possess genes that rate them high in the ranking compared to the one specimen of Aurochs.

Model based clustering

Model based clustering gives insight in the ancestry. Figure 4.1 shows the result of the calculations, assuming three different clusters: zebu (blue) north European cattle (green) and south/ European cattle. The aurochs specimen (the rightmost column of figure 4.1) contains elements from all three clusters. The pattern suggests zebu influence in the East-European (Podolian, Busha) but not in the Iberian clusters. From the domestication history this makes sense, since the East-European cattle has been in contact with Anatolian cattle, which underwent already zebu influence since 5000 BP.

Parentage check

Since Tauros-herds often have more mating bulls, it's important to have a good parentage check. The parentage of the first 100 offspring of the Tauros Programme has been checked with a 40 k SNP array. The 40 k analyses in a way are an overkill for the parentage check, but the same data will be useful to check the segregation of specific traits, once we know for which specific genes and traits we have to look.

The results of the heredity check are presented in Annex 5. From 126 out of the 130 sampled individuals it was possible to positively determine the identity of at least one of the parents. In most cases the mother was already known from observations in the field, but in other cases material of the mother was not available anymore. Most fathers were known as donors of artificial inseminations, but several fathers of important animals have not been sampled and remain (as yet) unknown. Once we will have sampled more individuals we will be able to positively identify the parentage of all Tauros offspring.

The parentage test revealed that in the herd of Keent nearly all matings were by the Maremanna x Pajuna bull (Manolo Uno, nr NL 896492389) and that a second bull in line – nr NL 896492390, one year younger – did not perform. In the other large area in the Netherlands (Kempen-Broek) the diversity of reproducing bulls is larger, because the area is split into several smaller areas each with a different bull and if available more bulls in one group.

Figure 4.1: Ancestry assignment of all the individuals with K=3[9]

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[9] Probably the White Park Cattle and Romanian Grey samples have been switched in this figure. This will be corrected in the final paper Maulik et.al., 2015.

5 The meaning of genetic research for the Tauros Programme

The genetic research is one of the pillars of the Tauros Programme. Our initial ideas were pretty straightforward: sample a fair number of Aurochs individuals and compare these with the breeds you want to use and already are using, and choose the most aurochs-like animals. Of course the road appeared bumpier than we expected with a major set-back our failure of analysing aurochs material. This approach in itself was not unrealistic however.

Meaning of the results

For the first time a genealogical table of aurochs and primitive cattle breeds, based on 770K SNP autosomal DNA, has been constructed (table 4.1). Although still preliminary, it is encouraging that the outcome of the research confirms our breeding programme so far. It roughly confirms that four of the breeds that do play a major role in our breeding programme are in the top 10 and six of our breeds are in the top 15. We expect to be able to draw more definite conclusions when more specimens of aurochs will have been analysed.

There are other primitive breeds we did not consider yet such as Ukranian Grey, Hungarian Steppe cattle, or the offspring of elder Iberian cattle breeds in South America (Criollo). Especially the latter could be very promising since, some of these herds stem directly from old Iberian breeds without too much external influence.

The speed of our research wasn’t as high as we hoped for initially. But the initial slow progress meant that we were able to include new insights such as Felius et. al. (2014) and Lenstra et. al. (2015). They provided the big picture of cattle domestication and the spread of genes. This detailed picture was earlier not available. We can now conclude that our results are in line with those two publications.

The strategy of combining Iberian and Podolian primitive breeds is in line with the outcome of the genetic research. By this strategy two routes of cattle-domestication and two different resources of ‘primitive’ genes are combined. The Iberian breeds have retained much genetic diversity, which was increased by the influence of African cattle with the African aurochs ancestry. In contrast, Podolian breeds have a different origin in the Balkans and may very well have picked up gene variants of European aurochs.

Next steps

Obviously, we will have to continue out investigations and also continue our international collaborations. Our plans are the following:

- At the short term, a phenotypical description of individuals as well as more practical guidelines for selection (based on the gross-list of breeding criteria). Now that the first 100 offspring have been analysed, we need to describe the most essential feature of the animals as well. We will now work out a practical programme to make measurements at individual animals. This will enable us to predict the development of animals in an early stage and to speed up the selection process;

- We will carry out whole genome sequencing of approximately 10 Tauros founder animals;
- Most essential will be genotypes of other aurochs individuals to assess the genetic variation within aurochs. This is being worked on by the research groups of MacHugh and Bradley and also on the University of Lyon;
- On the long term we need to have more insight in identity of the genes for relevant traits, so that we will be able to predict to a certain extent what the development of a young individual will be. This analysis will be performed by the pHD in his next years.

Since the Taurus Foundation has not the required resources, additional budgets will be necessary to carry out this research and to set priorities.

Glossary

- *Alleles*: Alternative forms of a DNA sequence
- *Autosomal chromosomes*: All chromosomes except the X and Y sex chromosomes.
- *CNV*: Copy number variation. A certain DNA segment can contain more copies of a gene, each copy not necessarily being a perfect copy. The number of copies can depend on the individual and thus is a polymorphism: the CNV.
- *Chromosome*: A long double-stranded and folded DNA molecule that contains genetic information and is passed to next generation
- *Diploid*: An organism with two copies of each chromosome, one from each parent.
- *DNA*: Deoxyribonucleic acid; the molecule that carries genetic information
- *Dominant*: A phenotype or its corresponding allele that in a heterozygote masks the presence of the alternative, recessive phenotype of the same locus in the heterozygote;
- *F1 generation* First-generation offspring of a cross;
- *F2 generation* Second-generation offspring of a cross;
- *Gene*: The fundamental unit of heredity; a specific and functional section of DNA within a chromosome that codes for a protein or RNA molecule
- *Genetic distance*: A statistic tool used to measure the differences in allele frequencies between species or breeds. The smaller the distance the more two breeds or species are alike. A standard tool in genetics is NEI genetics distance.
- *Genotype*: The genetic makeup of an individual; the allele combination at a given locus
- *Haplotype:* The combination of alleles in a chromosome, gene or mtDNA molecule is called a haplotype
- *Haplogroup*: A number of similar haplotypes is called a haplogroup. Haplotypes and haplogroups within species often have a geographic distribution indicating historic colonization-routes
- *Heterozygote*: An individual with two different alleles at a given gene or locus
- *Homozygote*: An individual with two identical alleles of a given gene or locus
- *Locus*: A specific location on a chromosome
- *mtDNA:* Mitochondrial DNA is outside the nucleus. It does not take part in the sexual reproduction, but is transmitted from mother to offspring.
- *mRNA:* Messenger RNA (mRNA) directs the synthesis of proteins
- *Phenotype*: The physical characteristics of an individual
- *Recessive*: A phenotype exhibited only when homozygous or the allele corresponding to this phenotype.
- *Sex chromosomes*: Chromosomes controlling the gender. In mammals these are the X and Y chromosomes with two X chromosomes (XX) in females and one X and one Y (XY) in males.
- *Sex linked*: phenotype controlled by a gene on one of the sex chromosomes

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Annex 1: LONGLIST OF 'ORIGINAL' BREEDS

Breed	Code	Country	Region
Alentejana	AL	Portugal	Iberian
Angler	AN	Germany	Dutch
Arouquesa	AR	Portugal	Iberian
Aurochs	AU	Britain	Bos primigenius
Berrenda en Colorado	BC	Spain	Iberian
Berrenda en negro	BN	Spain	Iberian
Boskarin	BK	Croatia	Balkan and Italy
Brown Swiss	BS	Switzerland	Alps
Busha	BU	Balkan region	Balkan and Italy
Cachea	CC	Portugal	Iberian
Caldela	CL	Portugal	Iberian
Cardena	CA	Spain	Iberian
Chianina	CH	Italy	Balkan and Italy
English Longhorn	EL	Britain	English
Fleckvieh	FL	Switzerland	Alps
Galloway	GA	Britain	English
Heck	HE	Germany	Heck
Jersey	JE	Jersey Island	Jersey
Kerry cattle	KC	Ireland	English
Lidia	LI	Spain	Iberian
Limia	LM	Spain	Iberian
Maremmana	MA	Italy	Balkan and Italy
Marondesa	MN	Spain	Iberian
Mirandesa	MI	Portugal	Iberian
MT	Maltese	Malta	Balkan and Italy
Nelore	NE	India	Bos Indicus
Pajuna	PA	Spain	Iberian
Podolica	PO	Ukraine	Balkan and Italy
Romenian grey	RO	Romania	Balkan and Italy
Sayaguesa	SA	Spain	Iberian
Scottish Highlander	HL	Scoland	English
Tudanca	TU	Spain	Iberian
White park	WP	Britain	English

Annex 2: Set with criteria for the Tauros Programme

Characteristics	Bull	Cow
Shoulder height	160 to 185 cm	145 to 150 cm
Overall build	• Narrow / slender • Forehand: dorsal protrusions of the vertebrae, attached to the back of the skull. This is needed for the muscles to carry a large head with horns • S-shaped back/spine curvature as a result of dorsal protrusions of the shoulder vertebrae to connect neck muscles • Square build regarding trunk length and leg length.	• Narrow / slender • • • • • S-shaped back/spine curvature • • • Square build regarding trunk length and leg length
Hind quarters	• Slender • Straight legs • Hip joint: sloping	• Slender • Straight legs • Hip joint: sloping (to facilitate natural calving ease)
Muscling	• No double muscling • Muscles can be somewhat pronounced, but functional • Neck is muscled • No typical meat cattle conformation	• No double muscling • Muscles can be somewhat pronounced, but functional
Coloration	• Calves are red • Coloration changes to black/dark brown after a few to six months • Selection against too lightly colored individuals • Lighter saddle on the back (shading in general) is permitted • Light eel stripe • White or light muzzle (can get less distinct when animal is older than 2 years) • Dark nose, no pink nose color permitted	• Calves are red • Red to brownish when mature • Selection against too dark or too lightly colored individuals; Cows should be lighter colored than bulls • Lighter saddle on the back (shading in general) is permitted • Can have a light eel stripe, but this is not mandatory • White or light muzzle • Dark nose, no pink nose color permitted
Legs	• Comparatively long legs. • Square build regarding trunk length and leg length.	• Comparatively long legs. • Square build regarding trunk length and leg length.
Head	• Long and slender. • Big in comparison with the rest of the body.	• Long and slender. • Big in comparison with the rest of the body.
Horns	• Length: 60 to 107 cm • Circumference on the base: 10 to 18 cm • Form: variable, but on average as follows: in the beginning growing somewhat backwards and upwards in an angle of 0 to 30 degrees, then in curving forward and inwards in an angle of 60 degrees in relation to the skull and finally upwards and outwards.	• Length: 40 to 70 cm • Circumference on the base: 7 to 10 cm • Form: variable, but on average as follows: in the beginning growing somewhat backwards and upwards in an angle of 0 to 30 degrees, then in curving forward and inwards in an angle of 60 degrees in relation to the skull and finally upwards and outwards.

Characteristics	**Bull**	**Cow**
Coat	• Single short coat in summer • Double longer coat in winter • Fast shedding of winter coat in spring	• Single short coat in summer • Double longer coat in winter • Fast shedding of winter coat in spring
Dew lap	Not overly present like in Zebu	Not overly present like in Zebu
Hump (neck)	No hump like in Zebu, except for functional dorsal protrusions of the vertebrae to connect neck muscles.	Not present like in Zebu
Udder		Small, hairy and hardly visible

Annex 3: Brief summary of the breeds involved

Maremmana 'Primitiva' (central Italy)

The Maremmana breed comes from Maremma, a prairie and coastal dune region in western central Italy. Maremmana cattle are adapted to wet and marshy areas, but do well in mountains as well. The Maremmana primitiva has strong aurochs features like the size, colour setting, thick horns, clear difference in colour between males and females and a natural herd structure. Long-legged, large cattle, with bulls reaching up to 170 cm wither height, swung-back line in bulls, caused by the strongly expressed shoulder area, extremely hardy. Maremmana primitiva are extremely hardy cattle and can survive well on poor forage. In Italy, the Maremmana live in natural semi-feral herds.

Pajuna (southern Spain)

The Pajuna is a rare cattle breed from Andalucía in Southern Spain, with a long-legged athletic body, allowing them to move around the terrain with great ease. Pajuna bulls can reach up to 165 cm shoulder height. The horns, especially of the bulls, are facing forwards and have a light colour with a dark tip. Bulls are dark brown to black with a light eel stripe, while the cows are reddish-brown with a long face. They are able to survive under harsh conditions and on poor forage. High in the Spanish Sierra Nevada they live mostly from thorny shrubs. And in the lowland valleys they are known to survive on semi-desert-like vegetation, if needed. The breed adapts easily to changing circumstances, which is also an ancient trait. In Andalucía they are frequently kept in larger areas, with a natural herd structure.

Limia (north-western Spain)

The Limia cattle are from Galícia, in northwestern Spain and are a large-sized breed as well. Limia cattle also have clear difference in coat colour and shoulder height between males and females. With their slender bodies they move with great ease, and they are able to survive on poor forage. Galícia is relatively cold and wet, so the animals are used to living in a wet country, with cold and snowy winters, like in the more northern parts of Europe.

Sayaguesa (north-central Spain)

The Sayaguesa or Zamorana is an old cattle breed from Sayago in the province of Zamora west of Salamanca in Spain. Traditionally, Sayaguesas were used for pulling ploughs, carts and wagons. It's a large-sized breed, with bulls weighing on average between 1000 and 1100 kg and cows between 650 and 700 kg. The bulls are black and sometimes still have a light eel stripe. The cows often are just slightly lighter than the bulls, since farmers select on black colour, but reddish-brown Sayaguesa cows still can be found. They also have the white coloured muzzle of the aurochs. The horns are aurochs-like and point forward, especially in the bulls. Sayaguesa is a long-legged breed with a curved backline and a long face. The breed is known to be hardy and robust and doesn't need any shelter or supplementary feeding in the Spanish dehesa-countryside. Their behaviour is watchful and shy, and the cows will strongly defend their calves.

Maronesa (northern Portugal)

Maronesa is a hardy breed from the Trás-os-Montes region in northern Portugal among wolves. Some lineages are very aurochs-like. The bulls are much larger than the cows, with a dark brown to black coat colour and a light eel stripe, a lightly coloured muzzle and very large horns. Maronesa cows are reddish-brown. The heads of bulls are large and they are well muscled with a curved backline from the strongly pronounced shoulder part. The horns are thick and point forward. Maronesas are used to harsh conditions with snowy winters and hot summers and able to live without any human management, with calves growing up successfully also with wolves around.

Annex 4: Overview of conducted research

The amount of research has been limited by the available budget. In the period 2010-2015 chronologically the following steps have been taken as part of the genetic research specifically oriented toward the Tauros Programme.

1. In 2010, as part of and funded by Wageningen University, department Animal Breeding and Genetics (ABG) the following research has been done:
   - The collection and genetic analysis of 30 original breeds (together with commercially interesting breeds such as Holstein-Frisian and MRY) with the 770K SNP array technology
   - The collection and analysis with the 770K SNP array of around 25 different museum specimens of aurochs;
2. In 2012 we tried via California University to obtain a complete genome sequence from a medieval Polish aurochs (funded by Taurus Foundation);
3. In 2013-2015 additional research carried out with the funding of the Liberty wildlife fund has been done:
   - Analyses of 100 crossbreds from the Tauros programme (inheritance check);
   - 770 k SNP analyses of additional breeds (24 individuals);

   Although this part of the research is financially not the most substantial, it is most relevant to the Tauros Programme, since without the inheritance check and the further analysis of the offspring generations, we will not be able to check if the Programme moves in the right direction;
4. In 2014-2015 the complete genomes of a number of important animals at the base of the Tauros Programme will be sequenced. This part of the research has been made possible by the funding by the Food-Valley programme of the Province of Gelderland with 50 % co-financing of the Taurus foundation;

Over the period 2014-2018 further research and analyses will take place, which was made possible by European funding of the EGS-ABG programme (PhD student) and will ultimately lead to practical recommendations for the breeding programme;

Annex 5: The results of the inheritance check

Results of the inheritance check. In some cases not enough genetic material was available, in that case the cell in this table is a blank. This will be solved in later analyses.

	rund	moeder	vader
1	545195012	376909631	53990031794
2	545195454	53990019996	53990031794
3	617711740	21107310624	53990052783
4	743694832	53990019995	53990031794
5	743695013	529492238	90807606401
6	745492740	421414280	53990031794
7	844298139	53990060193	Goffy
8	896492389	53990019995	Goffy
9	925696414	2110745436	896492389
10	925696483	522803091	31104103451
11	925696818	90104784771	896492389
12	925696832	21107310759	896492389
13	925696856	539900199995	896492389
14	925696902	71107310549	896492389
15	925696940	53990060204	896492389
16	925697082	896492341	50106187461
17	440854133	241297191	286761561
18	526991561	425202418	Bernardo
19	529492238	440854133	53990031819
20	535903296	425202502	Julio
21	545195005	533491768	53990031794
22	709292274	418536393	53990031794
23	709292298	323445106	53990031794
24	713403354	472702989	Jose
25	719994582	522603112	
26	719994607	433089850	896492389
27	743694919	418536393	896492389
28	745492841	418536362	53990031794
29	745492858	524990573	53990031794
30	815742026	414107057	514107127
31	887603460	522803091	Jose
32	925696337	60807600739	
33	925696438	414107057	
34	925696584	713403378	90105426310
35	925696894	323445106	896492390

36	925696926	50807262136	
37	925696957	80807262435	
38	925697020	20105427350	896492389
39	925697051	546492659	50106187461
40	925697107	709293107	50106187461
41	925697138	709293044	50106187461
42	925697145	721393551	50106187461
43	925697183	887603453	
44	933798324	472702989	
45	10807502791	50807618816	
46	20105427350	90104784771	
47	50807262136	60807600739	
48	90810072548	80807262140	
49	525590963	370304537	
50	526991390	284418076	
51	529492245	241297191	53990031819
52	533491814	370304520	53990031794
53	545195245	709292274	90807606401
54	545195461	479830100	53990052783
55	545195562	353842274	896492389
56	545195788	370304537	896492389
57	545195795	399720891	53990052783
58	545196008	883794304	53990052783
59	545196008	61105912075	53990052783
60	545196015	284949088	53990052783
61	545196022	472356731	53990052783
62	655303439	21107310624	53990052783
63	713403417	80807262457	
64	721393342	53990019995	limia-lacon
65	743694849	71107310549	31104103451
66	743694856	470099997	896492389
67	743694902	2110745436	31104103451
68	743694971	21107310759	31104103451
69	743694995	379111246	896492389
70	807502790	60807262148	
71	883794304	61105912075	53990052783
72	887603509	425202502	
73	896492341	376909631	53990031794
74	896492411	370304520	53990031794
75	925696320		896492389
76	925696344	479689812	896492389

77	925696351	527791195	896492389
78	925696382	379111260	896492389
79	925696399		896492389
80	925696421	497390343	896492389
81	925696445	379111246	896492389
82	925696452	745492997	896492389
83	925696469	470099911	896492389
84	925696577	738993793	90105426310
85	925696623	53990063275	90105426310
86	925696700	83148	90105426310
87	925696779	470099997	896492389
88	925696786	479689766	896492389
89	925696793	433089850	896492389
90	925696801	339089693	896492389
91	925696870	546492510	896492389
92	925696919	525590963	896492389
93	925696964	53990019996	90105426310
94	925696971	745492740	896492389
95	925696995	370304520	896492389
96	925697006	370304537	896492389
97	925697013	496590168	896492389
98	925697037	284418052	896492389
99	925697044	527791311	896492389
100	925697099	745492841	50106187461
101	925697121	896492341	50106187461
102	925697152	533491814	50106187461
103	925697152	896492411	50106187461
104	925697169	526991390	896492389
105	925697176	241297191	896492389
106	925697121	896492341	50106187461
107	925697152	533491814	50106187461
108	925697152	896492411	50106187461
109	925697169	526991390	896492389
110	925697176	241297191	896492389
111	20807502792	30807606416	
112	21107310624	91105237441	9716
113	21107310624	91105237441	9718
114	21107310624	91105237441	9814
115	30809822607	30807606416	
116	31104103451	81104103138	limon
117	61105912075	61103323901	lacon

118	90104784771	90104054854	PAJ23
119	53990052783	11GR148C007	9716
120	53990052783	11GR148C007	9718
121	53990052783	11GR148C007	9814
122	527791126	370304520	225690381
123	545194824	53990060204	53990031794
124	545194949	377628465	53990031794
125	545195601	284418076	53990052783
126	883794506	379111246	53990031794
127	887603561	425202502	Julio
128	896492396	241297191	53990031819
129	925697114	896492411	50106187461
130	60807262148	50807618816	