Lactase persistence and milk consumption in Europe: an interdisciplinary approach involving genetics and archaeology

Michela Leonardi

AG Palaeogenetik, Institute of Anthropology, Johannes Gutenberg University, Mainz, D
Human Evolutionary Ecology Group, Department of Anthropology, University College London, London, UK
m.leonardi@ucl.ac.uk

ABSTRACT – *The ability to digest milk during adulthood (lactase persistence) is a genetically determined trait present only in humans. Its origin and diffusion are correlated with the development of pastoralism and the consumption of fresh milk. This work will present the genetic and archaeological data that allow the reconstruction of the co-evolutionary process between dairying culture and lactase persistence, as well as a discussion of the chronology and the way lactase persistence spread in Europe.*

IZVLEČEK – *Sposobnost presnavljanja mleka v odrasli dobi (laktazna persistenca) je genetsko pogojena značilnost, navzoča le pri ljudeh. Njen izvor in razširitev sta povezana z razvojem pastirstva in uživanjem svežega mleka. V članku predstavljamo genetske in arheološke podatke, ki nam omogočajo rekonstrukcijo koevolucijskih procesov med mlečno-gospodarskimi kulturami in laktazno persistenco, poleg tega pa komentiramo kronologijo in način, kako se je laktazna persistenca širila v Evropo.*

KEY WORDS – *lactase persistence; gene-culture coevolution; European Neolithic; genetics; dairying*

Introduction

Mammals have the ability to digest lactose – the main sugar contained in milk – only until weaning is over. After that, there is a significant reduction in the production of lactase, the enzyme that allows the absorption of lactose. In humans, however, at least four genetic mutations have been associated with the production of lactase into adulthood (lactase persistence). The frequency of lactase persistent individuals varies significantly between and within continents, and in some cases even between neighbouring regions. In Europe the distribution of lactase persistent individuals follows a cline showing lower frequencies in the south, and higher frequencies, reaching as much as 98%, in the north.

The study of ancient DNA shows that the lactase persistence associated variant was absent or present in very low frequencies in most regions before and during the Neolithic (*Burger* et al. *2007; Lacan* et al. *2011; Malmström* et al. *2009; Plantinga* et al. *2012*). This result suggests that, before the beginning of animal husbandry, the European population was not able to drink milk during adulthood without suffering from very unpleasant and sometimes deleterious symptoms. After the domestication of cattle, sheep and goats, milk became available as a source of nutrition for adults, and the frequency of the lactase persistence-associated mutation increased rapidly in the population, reaching the present-day rates.

The origin and spread of lactase persistence is a very complex process that, to be understood well, must be considered within its archaeological, genetic and social context (*Gerbault* et al. *2011; Leonardi* et al. *2012*).

Genetics

The digestion of milk

Lactose is a disaccharide sugar that can be found in different percentages in the milk of almost all mammals apart from the platypus and some marine mammals (*Reich, Arnould 2007*). Lactose cannot be digested in its disaccharide form; to be assimilated, it has to be hydrolysed and separated into two monosaccharide sugars (glucose and galactose). This is performed in the mammal intestine with the enzyme lactase (lactase phlorizin hydrolase or LCT). The production of lactase in the small intestine is usually either already high at birth (*e.g.*, in humans; *Wang* et al. *1994*), or peaks a few days later (*e.g.*, in rodents; *Troelsen 2005*), and remains at more or less the same level until weaning is over, when it decreases significantly.

When this happens, the undigested lactose reaches the colon, where it is fermented by colonic bacteria. The glucose is then fermented involving the production of short chain fatty acids and gases, entailing osmotic effects. The result of this process is the appearance of very unpleasant symptoms such as bloating, chronic flatulence, diarrhoea and abdominal cramps (*Ingram* et al. *2009a*). The severity of symptoms can vary between individuals showing the same levels of lactase production (*Vonk* et al. *2003*), since colonic adaptation can lead to a better capacity to ferment lactose and a reduction in the production of hydrogen by the colonic microbiota (*Szilagyi* et al. *2002*).

In about 35% of adults worldwide, lactase is produced throughout their life, allowing them to drink milk at any age without experiencing any of the above-mentioned ill effects (*Ingram* et al. *2009a*).

The genetics of lactase persistence

Lactase persistence is inherited as a dominant Mendelian trait (*Ferguson, Maxwell 1967; Sahi* et al. *1973; Sahi, Launiala 1977*). A single gene located in chromosome 2, called LTC, codes for lactase. Within the neighbouring MCM6 gene, a region (intron 13) seems to have an enhancer action on the LCT gene (*Fang* et al. *2012; Jensen* et al. *2011; Lewinsky* et al. *2005; Olds* et al. *2011; Olds, Sibley 2003; Troelsen* et al. *2003*). Several different single nucleotide polymorphisms (SNPs) in this region show a strong association with lactase persistence, and their distribution is geographically structured. The first mutation found to be linked to this trait is a cytosine to thymine transition 13 910 nucleotides upstream

of the transcription initiation site of the lactase gene (–13 910 C/T) (*Enattah* et al. *2002*). The derived variant at this locus (–13910*T) is associated with lactase persistence throughout Europe (*Anagnostou* et al. *2009; Ingram* et al. *2009a; Itan* et al. *2010; Manco* et al. *2013; Nagy* et al. *2009; Sun* et al. *2007; Torniainen* et al. *2009*), Central Asia (*Heyer* et al. *2011*) and India (*Gallego Romero* et al. *2012*).

However, in Africa –13 910 C/T alone does not explain the observed pattern of lactase persistence distribution, and several other SNPs within the same genetic region appear to be associated with the ability to digest large quantities of raw milk by adults. The same is true of the Middle East and in Tibet (*Al-Abri* et al. *2012; Enattah* et al. *2008; Imtiaz* et al. *2007; Ingram* et al. *2007; Ingram* et al. *2009b; Peng* et al. *2012; Tishkof* et al. *2007*). For this reason, several independent origins for lactase persistence have been proposed (*Enattah* et al. *2008; Ingram* et al. *2007; Peng* et al. *2012; Tishkoff* et al. *2007*).

Lactase persistence in modern worldwide populations

The frequency of lactase persistent individuals varies significantly between geographical regions (*Itan* et al. *2010*). In Eastern Asia and in Native American populations a small percentage of the population is persistent (*Itan* et al. *2010*). In Africa, the distribution is irregular, with frequencies that can reach as much as 92% in pastoralist populations, but can fall as low as 0.02% in non-pastoralist human groups (for a table listing the frequencies in a great number of global populations see http://www.ucl.ac.uk/mace-lab/resources/ glad, partially published in *Itan* et al. *2010*). A similar pattern can be observed in the Arabian Peninsula and in Jordan, with relevant differences between Bedouin and non-Bedouin neighbouring groups (*Al-Abri* et al. *2012; Ingram* et al. *2009a*). In the Indian subcontinent, however, a clinal pattern can be detected, declining from northwest to south-east (*Gallego Romero* et al. *2012; Ingram* et al. *2009a; Itan* et al. *2010*).

Also in Europe, a similar cline with frequencies increasing from south to north can be observed. In the northern part of the continent almost all the sampled individuals are lactase persistent, with frequencies ranging between 96% and 83% in Finland, Denmark, Ireland and the United Kingdom; in the Mediterranean area, the opposite trend can be observed (*Itan* et al. *2010; Manco* et al. *2013*).

Archaeology

Before exploring the origin and spread of lactase persistence in Europe in more detail, it is necessary to consider the cultural and archaeological context related to the beginning of milk consumption in Europe; this will be summarised in the next two sections.

The Mesolithic-Neolithic transition in Europe

The so-called Neolithic revolution marks the transition between a lifestyle based on hunting and gathering to one based on food production through cultivation and animal management and exploitation. The transition from the Palaeolithic-Mesolithic to the Neolithic entailed many different changes, not only related to material culture (*e.g.*, the acquisition of pottery) and knowledge of new skills (*e.g.*, those related to animal and plant domestication) but also to the social structure of the human groups (*e.g.*, sedentism and a different redistribution of wealth).

The first Neolithic cultures seem to have developed around the Fertile Crescent some 12 000 years ago, and then to have spread to neighbouring regions, including Europe, during the following few thousand years (*Flannery 1973*). Two opposite models have been proposed to reconstruct the way in which Neolithic cultures developed and spread in Europe.

The demic diffusion model suggests that the Neolithic culture and lifestyle spread from the Near East into Europe through the migration of farmers/agriculturalists (and possibly domesticates) with no substantial admixture with local hunter-gatherer populations (*Ammerman, Cavalli-Sforza 1984*). On the other hand, **the cultural diffusion model** theorises a step-by-step process whereby local Mesolithic groups learnt new skills from neighbouring Neolithic populations, without a replacement of people (*Zvelebil, Zvelebil 1988.574–583*). The more recent studies suggest that a more complex intermediate model involving a succession of migration phases interleaved by local admixture could be a better representation of what actually happened (*Whittle, Cummings 2007*). Genetic data have been used in order to differentiate between the two hypotheses, but the subject is still debated (*Barbujani 2012; Thomas* et al. *2013*).

The pattern of modern non-recombinant diversity in Europe has in some cases been interpreted as supporting the demic diffusion model (*Balaresque* et al. *2010; Barbujani, Bertorelle 2001; Battaglia* et al. *2009; Dupanloup* et al. *2004; Simoni* et al. *2000a; 2000b; Torroni* et al. *2001*) and in others as supporting a major Palaeolithic ancestry for extant lineages (*Pala* et al. *2012; Richards* et al. *2000; Scozzari* et al. *2001; Semino* et al. *2000*).

On the other hand, it must be acknowledged that a rough description of the data can lead to misinterpretation when different hypotheses are not statistically tested through explicit simulations (*Barbujani 2000; Barbujani* et al. *1998; Francois* et al. *2010; Novembre, Stephens 2008; Pinhasi* et al. *2012; Simoni* et al. *2000b*). Simulation studies on this subject have also led to contradictory results (*Barbujani* et al. *1995; Belle* et al. *2006; Chikhi* et al. *1998; Chikhi* et al. *2002; Currat, Excoffier 2005*).

The main problem related to this kind of analysis is that, probably at this stage, even computer simulations are not able to take into account the many different variables that play a role in shaping the genetic patterns of populations (*Pinhasi* et al. *2012*). A very recent article has highlighted some of these difficulties and tends to support a complex model of cultural diffusion affected by range contractions (*Arenas* et al. *2013*).

Ancient DNA can make an important contribution to the solution of this problem (*Pinhasi* et al. *2012*). Several studies have analysed mitochondrial DNA from late hunter-gatherers and/or Early Neolithic samples from different European regions (*Hervella* et al. *2012*): from central Europe (*Bramanti* et al. *2009*), France (even if from a limited number of samples; *Deguilloux* et al. *2011*), Iberia (*Sanchez-Quinto* et al. *2012*) and Scandinavia (*Malmstrom* et al. *2009; Skoglund* et al. *2012*). These studies suggest a local discontinuity with modern European populations. Data from Hungary (*Guba* et al. *2011*) suggest the same pattern, but the chronological attribution of some samples has been questioned (*Banffy* et al. *2012*). In Denmark, on the contrary, continuity with the pre-Neolithic population has been proposed (*Melchior* et al. *2010*).

The role of milk consumption in the Neolithic revolution

Before the Neolithic, milk was available only during the first years of life (*i.e.* breastfeeding); only after the beginning of animal domestication did it become a possible source of nourishment for adults. Lactase persistence, even if already present in some individuals, would have been of no utility until the beginning of the human management of cattle, sheep and

goats. The earliest evidence of milk related animal domestication appear in the Euphrates valley between 10 700 and 10 500 BP, and this is followed by a distribution to Eastern and Central Europe during the following millennia (*Vigne 2011; Zeder 2008*). Domesticated goats and sheep were brought to Europe from the Near East, but for cattle a separate domestication process in Europe would have been possible, although recent simulation studies support a single domestication process in the Middle East (*Bollongino* et al. *2012*).

During the last few decades, there has been a dramatic change in opinion about the importance of milk consumption at the beginning of the Neolithic. The 'Secondary Product Revolution' model has hypothesised that animals were domesticated to exploit so-called primary products, materials made available with the death of the animal (meat, bone, horn, leather *etc.*), and only subsequently was attention directed towards products that can be collected while the animal is alive (wool, milk, labour *etc.*) (*Sherratt 1981*).

However, recent interdisciplinary studies have demonstrated that milk was used from the beginning of animal domestication. Through an analysis of the age and sex ratio in archaeological assemblages of animal bones (*i.e.* kill-off profiles) from the Early Neolithic, it has been possible to show that the exploitation of cattle, sheep and goats was aimed at the production of milk, and not only meat, in both Mediterranean Europe and the Middle East (*Vigne, Helmer 2007; Vigne 2008*).

Moreover, the analysis of organic residues in pottery allows us to recognise whether ceramics were used to ferment milk (*Dudd, Evershed 1998*). Through this method it has been possible to demonstrate that milk exploitation and cheese production started from the beginning of the Neolithic both in the Middle East and in Europe (*Copley* et al. *2005; Craig* et al. *2005; Craig* et al. *2005; Evershed 2008; Salque* et al. *2013*).

The origins of lactase persistence in Europe

The mutation associated with lactase persistence in Europe (−13 910*T) is relatively recent. Estimates based on the method of long-range haplotype conservation suggest its origin between 2188 and 20 650 years ago (*Bersaglieri* et al. *2004*) while an analysis of the variation in closely linked micro-satellites has dated it to a period between 7450 and 12 300 years ago (*Coelho* et al. *2005*). It is interesting to consider that the dates estimated for the origin of one of the African variants (14 010*C) are similar, even if slightly more recent (*Tishkoff* et al. *2007*).

Given the scenario presented, one of the most interesting questions that has been addressed during the last decade is whether the −13 910*T variant was already present in Europe in significant frequencies at the beginning of the Neolithic (*e.g.*, due to random mutation and the effects of genetic drift) or if the spread of the mutation associated with lactase persistence was purely the result of selection acting after the beginning of animal domestication, when milk became available as a nutritional source throughout the entire life of the individuals. The advent and further development of technologies allowing the sequencing of DNA extracted from archaeological specimens has helped answer this question.

The first study to address this issue was performed on a set of samples from Central Europe (Germany, Hungary, Lithuania and Poland) dating from the Mesolithic (one individual) to the Early Neolithic period (eight individuals) (*Burger* et al. *2007*). All of them were homozygotes for the ancestral allele, and therefore unable to digest fresh milk as adults. The authors performed statistical analyses that showed that the frequencies of the derived alleles must have been very low, if not nil, in order to have such a pattern in the sample.

More recently, several studies have been carried out in different regions of Europe clarifying this assertion (*Burger, Thomas 2011; Linderholm 2011*). In France, the mentioned SNP has been typed in 26 skeletons dated to the end of the Neolithic, before the beginning of the Bell-Beaker Culture, and all of these were homozygotes for the C variant (*Lacan* et al. *2011*). In southern Scandinavia only one out of ten Middle Neolithic hunter-gatherers carried the derived allele, and was heterozygote (*Malmström* et al. *2009*).

In contrast, in northern Spain, out of 26 Middle Neolithic individuals, seven (five of them homozygotes) carried the T allele (*Plantinga* et al. *2012*). The authors tend to explain this difference in frequencies, compared to the other European samples, as the effect of genetic drift rather than the result of natural selection.

Additionally, some more recent samples, from the Middle Ages, have been analysed. A single individual

from Germany was lactase persistent, being heterozygous (*Burger* et al. *2007*), and out of 23 samples from Hungary only three carried the derived haplotype (*Nagy* et al. *2011*). It has nevertheless to be born in mind that for periods such as the Middle Ages high levels of gene flow and important migrations occurred in Europe, and this must be considered when interpreting such data (*Nagy* et al. *2011; Reich, Arnould 2007*).

Selection on lactase persistence

The above-mentioned data support a massive increase of LP frequency from virtually 0 to close to 100% in Northern Europe in a few thousand years, which is a very short period when considering the evolution of our species. This evidence suggests strong natural selection acting on this locus: the coefficient of selection has been estimated as reaching almost 0.2 in Northern European populations (*Bersaglieri* et al. *2004*). Such a strong indication of selection poses the problem of understanding why drinking fresh milk had such a significant selective advantage.

It is important to consider that the intake of small quantities of fresh milk does not always cause unpleasant symptoms in non-persistent individuals, and that when milk is processed or fermented, such as in yogurt or cheese, the amount of lactose decreases and the consumption of these products does not cause symptoms to non-persistent individuals (*Hammer* et al. *1998*). Moreover, as already discussed, gut flora adaptation can in some cases allow individuals to avoid the symptoms of lactose malabsorption (*Szilagy* et al. *2002*).

The evidence of cheese production during the Early Neolithic mentioned above has a special meaning. It has already been discussed that lactase persistence was very rare or absent during the Neolithic, and this would not allow human groups to drink fresh milk. Processed milk, on the other hand, can be digested by non-persistent individuals without unpleasant symptoms. The production of cheese at the beginning of the Neolithic suggests that at that time human groups may have already recognised the high nutritional benefits available from milk, and, in order to digest it, they processed it. It can be presumed, therefore, that the reason lactase persistence spread and became so common in Europe is not to be found in the simple nutritional benefits of consuming fresh milk.

The observations above raise a question: why is lactase persistence so strongly selected if non-persistent individuals can consume milk by processing it, thereby gaining many of milk's nutritional benefits? Several possible explanations have been proposed, but it is still under debate as whether any is convincing enough to justify levels of positive selection as high as those observed. As previously discussed, high frequencies of lactase persistence tend to correlate with a traditionally pastoralist lifestyle or with high consumption of fresh milk, even if this is not always the case (*Ingram* et al. *2009b*). This evidence could be the result of two quite different scenarios.

The reverse-cause argument (*McCracken 1971*): after its first appearance, a mutation associated to lactase persistence could have grown in frequency within one or several small human groups only because of genetic drift. Milk drinking could then have been adopted since the group would have been able to tolerate it.

Gene culture co-evolution (*McCracken 1971. 497–517, Simoons 1970.695–710*): lactase persistence could have been positively selected in dairying populations, which had access to fresh milk throughout life.

As already noted in the previous section, ancient DNA data support the second hypothesis. During the Neolithic, when dairying practices were already established, lactase persistence appears to be virtually absent, or present in very low frequencies in Europe. It is also important to consider that the random independent origin and then the increase of the frequencies of different lactase persistence-associated alleles due to the random fluctuation (genetic drift) in different regions would be a very complex and unlikely scenario, for which no possible explanation is available at the moment.

The calcium assimilation hypothesis (*Flatz, Rotthauwe 1973*): exposure to sunlight allows human skin to produce vitamin D, which is an element of great importance for the absorption of calcium in the bones. When vitamin D, taken in through a diet rich in fish or produced thanks to exposure to the sun, is not sufficient, bone development can be seriously compromised. In addition to other nutrients, milk contains small amounts of vitamin D and large amounts of calcium. The frequency of lactase persistence in Europe correlates with latitude and sunlight, and this evidence suggests a cause-effect relationship between the two. In Northern Europe, it is possible to observe a transition from a diet rich in fish and marine organisms (rich in vitamin D) during

the Mesolithic to one based mainly on cereals (poor in vitamin D) in the Late Neolithic (*Eriksson* et al. *2008; Lidén, Eriksson 2007; Lidén* et al. *2004*). The Meso-Neolithic transition has been shown by many scholars to be associated with a deterioration in health (*Cohen 2008; Eriksson* et al. *2008; Eshed* et al. *2010; Hershkovitz, Gopher 2008; Larsen 1995*). Drinking milk would have helped populations living where sunlight is low to avoid rickets and similar problems related to such a nutritional change.

Adaptation to arid environments (*Cook, al-Torki 1975.135–136*): in a normal situation, raw milk and cheese could provide the same nutritional benefits, but in arid environments milk could be an uncontaminated source of fluid, while lactose intolerance related symptoms (mainly diarrhoea) could lead to dehydration and, eventually, death. Nevertheless, this hypothesis is not easily applicable to Europe, where the climate is temperate.

A statistical test was performed to see whether lactase persistence was more likely to be correlated with dairying practice, calcium assimilation or adaptation to arid environments (*Holden, Mace 1997. 605–628*). The best correlation was obtained between the ability to digest milk and pastoralism. This study suggests, in accordance with the palaeogenetic data, that pastoralism was more probably adopted before lactase persistence arose or became frequent.

The spread of lactase persistence through Europe

The pattern of genetic diversity that can be observed in a population is the result of many different biological and social processes that contribute to shaping it during its history: random mutation, natural selection, genetic drift (and, consequently, demography), social factors influencing mating choices (*e.g.*, social structuring) *etc*.

Computer simulations are very powerful instruments for testing various hypotheses through models with different degrees of complexity. In this way, the most important factors playing a role in shaping the diversity observed can be explored both in a computationally effective way, removing the confounding effect of minor elements, and within a realistic framework integrating all the information that appears to be relevant to the problem. Computer simulations have been applied to investigate the evolution of LP since the late 1980s (*Aoki 1986*). As already mentioned, lactase persistence is not entirely correlated

with pastoralism and milk drinking, since some individuals (or human groups) are able to drink milk without being lactase persistent. Aoki tried to test if this could be the result of a process of gene-culture co-evolution and, by using computer simulations, he confirmed that this is the case. Moreover, his study showed that the incomplete correlation could be easily linked to the stochastic nature of the process.

Two recent studies have reached different results when simulating the spread of lactase persistence in Europe. As already discussed, it is still debated whether the beginning of the Neolithic in Europe was linked to the migration of people from the Middle East or to simple cultural transmission of skills and techniques from agriculturalist populations to neighbouring hunter-gatherers. The colonisation and expansion in Europe by farmer populations could have had an important impact on the diffusion of the lactase persistence-associated allele, since some demographic processes could mimic the effects of selection (*Klopfstein* et al. *2006*). The impact of demographic effects and differential selection based on latitude have been taken into account in a recent study simulating the spread of lactase persistence in Europe (*Gerbault* et al. *2009*). The authors tested two different scenarios for the spread of the Neolithic in Europe (the demic and cultural diffusion models). On the basis of the dates for the beginning of the Neolithic in each region, they simulated the evolution of the frequencies of the lactase persistence associated allele through time. Selection was incorporated in three different ways: constant throughout the continent, increasing towards the north (to test the calcium assimilation hypothesis) or higher in central European Early Neolithic (Linearband Ceramic) populations.

The results show that the present-day frequencies of milk digesters in southern Europe could be due to genetic drift linked to the arrival of Neolithic farmers from the Near East, but selection is required to reproduce the modern frequencies observable in the northern part of the continent. The authors then support the demic diffusion model associated with the calcium assimilation hypothesis.

Spatially explicit simulations were also applied to the same subject (*Itan* et al. *2009*). After creating a geographical background as close to Europe as possible, they modelled the evolution of the lactase persistence associated variant in three humans groups: hunter-gatherers (already present in the continent prior to the beginning of the simulations, 9000 years

ago), dairying farmers, and non-dairying farmers. Food producers were allowed to reach a higher population density, and they spread from the Middle East towards Europe. Gene flow between groups, long-distance migrations and the cultural diffusion of subsistence practice were also included in the model. Positive selection acted only on dairying farmers. The best simulations were chosen on the basis of the fit with the arrival time of agriculture and modern frequencies of lactase persistence in 12 different locations. The method used to analyse the results allowed the estimation of migration rates, selection coefficients and time and geographical coordinates for the beginning of selection on lactase persistence; the best fit was reached when selection started in a region between the Balkans and Central Europe, from 6256 to 8683 years BP, and differential selection in Northern Europe was not necessary to reach modern frequencies. The times and regions mentioned are in great agreement with the development of the Linearband Ceramic culture (*Pavúk 2005*).

An analysis of the differences between the two mentioned studies can be found in Leonardi *et al.* (*2012*). The simulation model used in Gerbault *et al.* (*2009*) is less complex than that used in Itan *et al.* (*2009*) in several ways. The model from the former that can be better compared with the latter is the so-called LBK scenario, where selection is higher only for the descendants of LBK groups. In this model, the frequencies of lactase persistence in Northern European populations do not reach present-day values, showing that higher levels of selection only in LBK-related populations is not enough to reconstruct in a satisfactory way the modern distribution of lactase persistence in Europe. Since gene flow and long-distance migrations between populations are not explicitly modelled, this result does not contradicts the findings of Itan *et al.* (*2009*) but suggests that selection was not constant through time and space, a hypothesis that has not been rejected by the latter study, where the selection coefficient was constant.

Conclusion

Lactase persistence is an amazing example of gene-culture co-evolution. The modern-day pattern of diversity at this locus in Europe is the result of the interaction of a large number of factors such as physiology, genetics, demography, migrations of people, social structuring, and cultural contact. The study of such a complex subject must start from a very careful analysis of the archaeological, historical and biological contexts and should be performed using methods that allow as far as possible integrations of the different types of information available. During the last decade, the increase of computational power coupled with more effective sequencing techniques has led to the possibility to simulate *in silico* more models, and to reconstruct with better precision the history of our and other species. A better understanding of the origin and spread of lactase persistence in Europe will definitely pass along this path.

∴

References

Al-Abri A. R., Al-Rawas O., Al-Yahyaee S., Al-Habori M., Al-Zubairi A. S. and Bayoumi R. 2012. Distribution of the lactase persistence-associated variant alleles –13 910* T and –13 915* G among the people of Oman and Yemen. *Human Biology 84: 271–286.*

Ammerman A. J., Cavalli-Sforza L. L. 1984. *The Neolithic transition and the genetics of populations in Europe.* Princeton University Press. Princeton.

Anagnostou P., Battaglia C., Coia V., Capelli C., Fabbri C., Pettener D., Destro-Bisol G. and Luiselli D. 2009. Tracing the distribution and evolution of lactase persistence in Southern Europe through the study of the T(–13 910) variant. *American Journal of Human Biology 21: 217–219.*

Aoki K. 1986. A stochastic model of gene-culture coevolution suggested by the "culture historical hypothesis" for the evolution of adult lactose absorption in humans. *Proceedings of the National Academy of Sciences USA 83: 2929–2933.*

Arenas M., Francois O., Currat M., Ray N. and Excoffier L. 2013. Influence of admixture and paleolithic range con-

tractions on current European diversity gradients. *Molecular Biology and Evolution 30: 57–61*.

Balaresque P., Bowden G. R., Adams S. M., Leung H. Y., King T. E. *et al.* 2010. A predominantly neolithic origin for European paternal lineages. *PLoS Biology 8(11): e1000285*.

Banffy E., Brandt G. and Alt K. W. 2012. 'Early Neolithic' graves of the Carpathian Basin are in fact 6000 years younger-appeal for real interdisciplinarity between archaeology and ancient DNA research. *Journal of Human Genetics 57: 467–469; author reply 470–461*.

Barbujani G. 2000. Geographic patterns: how to identify them and why. *Human Biology 72: 133–153*.

2012. Human genetics: message from the Mesolithic. *Current Biology 22: R631–633*.

Barbujani G., Bertorelle G. 2001. Genetics and the population history of Europe. *Proceedings of the National Academy of Sciences USA 98: 22–25*.

Barbujani G., Bertorelle G. and Chikhi L. 1998. Evidence for Paleolithic and Neolithic gene flow in Europe. *American Journal of Human Genetics 62: 488–492*.

Barbujani G., Sokal R. R. and Oden N. L. 1995. Indo-European origins: a computer-simulation test of five hypotheses. *American Journal of Physical Anthropology 96: 109–132*.

Battaglia and 17 authors 2009. Y-chromosomal evidence of the cultural diffusion of agriculture in Southeast Europe. *European Journal of Human Genetics 17: 820–830*.

Belle E. M., Landry P. A. and Barbujani G. 2006. Origins and evolution of the Europeans' genome: evidence from multiple microsatellite loci. *Proceedings of the Royal Society B. Biological Sciences 273: 1595–1602*.

Bersaglieri T., Sabeti P. C., Patterson N., Vanderploeg T., Schaffner S. F., Drake J. A., Rhodes M., Reich D. E. and Hirschhorn J. N. 2004. Genetic signatures of strong recent positive selection at the lactase gene. *American Journal of Human Genetics 74: 1111–1120*.

Bollongino R., Burger J., Powell A., Mashkour M., Vigne J. D. and Thomas M. G. 2012. Modern taurine cattle descended from small number of near-eastern founders. *Molecular Biology and Evolution 29: 2101–2104*.

Bramanti B. and 15 authors. 2009. Genetic Discontinuity Between Local Hunter-Gatherers and Central Europe's First Farmers. *Science 236: 137–140*.

Burger J., Kirchner M., Bramanti B., Haak W. and Thomas M. G. 2007. Absence of the lactase-persistence-associated allele in early Neolithic Europeans. *Proceedings of the National Academy of Sciences USA 104: 3736–3741*.

Burger J. and Thomas M. G. 2011. The palaeopopulation-genetics of humans, cattle and dairying in Neolithic Europe. In R. Pinhasi, J. T. Stock (eds.), *Human Bioarchaeology of the Transition to Agriculture*. John Wiley & Sons. Ltd. Chichester: 369–384.

Chikhi L., Destro-Bisol G., Bertorelle G., Pascali V. and Barbujani G. 1998. Clines of nuclear DNA markers suggest a largely Neolithic ancestry of the European gene pool. *Proceedings of the National Academy of Sciences USA 95: 9053–9058*.

Chikhi L., Nichols R. A., Barbujani G. and Beaumont M. A. 2002. Y genetic data support the Neolithic demic diffusion model. *Proceedings of the National Academy of Sciences USA 99: 11008–11013*.

Coelho M., Luiselli D., Bertorelle G., Lopes A. I., Seixas S., Destro-Bisol G. and Rocha J. 2005. Microsatellite variation and evolution of human lactase persistence. *Journal of Human Genetics 117: 329–339*.

Cohen M. N. 2008. Implications of the NDT for world wide health and mortality in prehistory. In J.-P. Bocquet-Appel, O. Bar-Yosef (eds.), *The Neolithic Demographic Transition and its Consequences*. Springer. New York: 481–500.

Cook G. C., al-Torki M. T. 1975. High intestinal lactase concentrations in adult Arabs in Saudi Arabia. *British Medical Journal 3: 135–136*.

Copley M. S., Berstan R., Mukherjee A. J., Dudd S. N., Straker V., Payne S. and Evershed R. P. 2005. Dairying in Antiquity III. Evidence from absorbed lipid residues dating to the British Neolithic. *Journal of Archaeological Science 32: 523–546*.

Craig O. E., Chapman J., Heron C., Willis L. H., Bartosiewicz L., Taylor G., Whittle A. and Collins M. 2005a. Did the first farmers of central and eastern Europe produce dairy foods? *Antiquity 79(306): 882–894*.

Craig O. E., Taylor G., Mulville J., Collins M. and Parker Pearson M. 2005b. The identification of prehistoric dairying activities in the western Isles of Scotland: an integrated biomolecular approach. *Journal of Archaeological Science 32: 91–103*.

Currat M., Excoffier L. 2005. The effect of the Neolithic expansion on European molecular diversity. *Proceedings of the Royal Society B. Biological Sciences 272: 679–688*.

Deguilloux M. F., Soler L., Pemonge M. H., Scarre C., Joussaume R. and Laporte L. 2011. News from the west: ancient DNA from a French megalithic burial chamber. *American Journal of Physical Anthropology 144: 108–118*.

Dudd S. N., Evershed R. P. 1998. Direct demonstration of milk as an element of archaeological economies. *Science 282: 1478–1481*.

Dupanloup I., Bertorelle G., Chikhi L. and Barbujani G. 2004. Estimating the impact of prehistoric admixture on the genome of Europeans. *Molecular Biology and Evolution 21: 1361–1372*.

Enattah N. S. and 20 authors. 2008. Independent introduction of two lactase-persistence alleles into human populations reflects different history of adaptation to milk culture. *American Journal of Human Genetics 82: 57–72*.

Enattah N. S., Sahi T., Savilahti E., Terwilliger J. D., Peltonen L. and Jarvela I. 2002. Identification of a variant associated with adult-type hypolactasia. *Nature Genetics 30: 233–237*.

Eriksson G., Linderholm A., Fornander E., Kanstrup M., Schoultz P., Olofsson H. and Liden K. 2008. Same Island, different diet: Cultural evolution of food practice on Oland, Sweden, from the Mesolithic to the Roman period. *Journal of Anthropological Archaeology 27: 520–543*.

Eshed V., Gopher A., Pinhasi R. and Hershkovitz I. 2010. Paleopathology and the origin of agriculture in the Levant. *American Journal of Physical Anthropology 143: 121–133*.

Evershed R. 2008. Organic residue analysis in archaeology: the archaeological biomarker revolution. *Archaeometry 6: 895–924*.

Fang L., Ahn J. K., Wodziak D. and Sibley E. 2012. The human lactase persistence-associated SNP –13 910*T enables in vivo functional persistence of lactase promoter-reporter transgene expression. *Human Genetics 131: 1153–1159*.

Ferguson A., Maxwell J. D. 1967. Genetic aetiology of lactose intolerance. *Lancet 2: 188–190*.

Flannery K. V. 1973. The Origins of Agriculture. *Annual Review of Anthropology 2(1): 271–310*.

Flatz G., Rotthauwe H. W. 1973. Lactose nutrition and natural selection. *Lancet 2: 76–77*.

Francois O., Currat M., Ray N., Han E., Excoffier L. and Novembre J. 2010. Principal component analysis under population genetic models of range expansion and admixture. *Molecular Biology and Evolution 27: 1257–1268*.

Gallego Romero I. and 16 authors. 2012. Herders of Indian and European cattle share their predominant allele for lactase persistence. *Molecular Biology and Evolution 29: 249–260*.

Gerbault P., Liebert A., Itan Y., Powell A., Currat M., Burger J., Swallow D. M. and Thomas M. G. 2011. Evolution of lactase persistence: an example of human niche construction. *Philosophical Transaction of the Royal Society B Biological Sciences 366: 863–877*.

Gerbault P., Moret C., Currat M. and Sanchez-Mazas A. 2009. Impact of selection and demography on the diffusion of lactase persistence. *PLoS One 4: e6369*.

Guba Z., Hadadi E., Major A., Furka T., Juhasz E., Koos J., Nagy K. and Zeke T. 2011. HVS-I polymorphism screening of ancient human mitochondrial DNA provides evidence for N9a discontinuity and East Asian haplogroups in the Neolithic Hungary. *Journal of Human Genetics 56: 784–796*.

Hammer J., Hammer K. and Kletter K. 1998. Lipids infused into the jejunum accelerate small intestinal transit but delay ileocolonic transit of solids and liquids. *International Journal on Gastroenterology and Hepatology (Gut) 43: 111–116*.

Hershkovitz I. and Gopher A. 2008. Demographic, biological and cultural aspects of the Neolithic Revolution: a view from the southern Levant. In J.-P. Bocquet-Appel, O. Bar-Yosef (eds.), *The Neolithic Demographic Transition and its Consequences*. Springer. New York: 441–479.

Hervella M., Izagirre N., Alonso S., Fregel R., Alonso A., Cabrera V. M. and de la Rua C. 2012. Ancient DNA from hunter-gatherer and farmer groups from Northern Spain supports a random dispersion model for the Neolithic expansion into Europe. *PLoS One 7: e34417*.

Heyer E., Brazier L., Segurel L., Hegay T., Austerlitz F., Quintana-Murci L., Georges M., Pasquet P. and Veuille M. 2011. Lactase persistence in central Asia: phenotype, genotype, and evolution. *Human Biology 83: 379–392*.

Holden C., Mace R. 1997. Phylogenetic analysis of the evolution of lactose digestion in adults. *Human Biology 69: 605–628*.

Imtiaz F., Savilahti E., Sarnesto A., Trabzuni D., Al-Kahtani K., Kagevi I., Rashed M. S., Meyer B. F. and Jarvela I. 2007. The T/G 13 915 variant upstream of the lactase gene (LCT) is the founder allele of lactase persistence in an urban Saudi population. *Journal of Medical Genetics 44: e89*.

Ingram C. J., Elamin M. F., Mulcare C. A., Weale M. E., Tarekegn A., Raga T. O., Bekele E., Elamin F. M., Thomas M. G., Bradman N. and Swallow D. M. 2007. A novel polymorphism associated with lactose tolerance in Africa: multiple causes for lactase persistence? *Human Genetics 120: 779–788*.

Ingram C. J., Mulcare C. A., Itan Y., Thomas M. G. and Swallow D. M. 2009a. Lactose digestion and the evolutionary genetics of lactase persistence. *Human Genetics 124: 579–591*.

Ingram C. J., Raga T. O., Tarekegn A., Browning S. L., Elamin M. F., Bekele E., Thomas M. G., Weale M. E., Bradman N. and Swallow D. M. 2009b. Multiple Rare Variants as a Cause of a Common Phenotype: Several Different Lactase Persistence Associated Alleles in a Single Ethnic Group. *Journal of Molecular Evolution 69(6): 579–588*.

Itan Y., Jones B. L., Ingram C. J., Swallow D. M. and Thomas M. G. 2010. A worldwide correlation of lactase persistence phenotype and genotypes. *BMC Evolutionary Biology 10: 36*.

Itan Y., Powell A., Beaumont M. A., Burger J. and Thomas M. G. 2009. The origins of lactase persistence in Europe. *PLoS Computational Biology 5: e1000491*.

Jensen T. G., Liebert A., Lewinsky R., Swallow D. M., Olsen J. and Troelsen J. T. 2011. The –14 010*C variant associated with lactase persistence is located between an Oct–1 and HNF1alpha binding site and increases lactase promoter activity. *Human Genetics 130: 483–493*.

Klopfstein S., Currat M. and Excoffier L. 2006. The fate of mutations surfing on the wave of a range expansion. *Molecular Biology and Evolution 23: 482–490*.

Lacan M., Keyser C., Ricaut F. X., Brucato N., Duranthon F., Guilaine J., Crubezy E. and Ludes B. 2011. Ancient DNA reveals male diffusion through the Neolithic Mediterranean route. *Proceedings of the National Academy of Sciences USA 108: 9788–9791*.

Larsen C. S. 1995. Biological changes in human populations with agriculture. *Annual Review of Anthropology 24: 185–213*.

Leonardi M., Gerbault P., Thomas M. G. and Burger J. 2012. The evolution of lactase persistence in Europe. A synthesis of archaeological and genetic evidence. *International Dairy Journal 22: 88–97*.

Lewinsky R. H., Jensen T. G., Moller J., Stensballe A., Olsen J. and Troelsen J. T. 2005. T–13 910 DNA variant associated with lactase persistence interacts with Oct–1 and stimulates lactase promoter activity in vitro. *Human Molecular Genetics 14: 3945–3953*.

Lidén K., Eriksson G. 2007. Walking on the wild side: on cultural diversity and the Pitted Ware Culture along the Swedish east coast during the Middle Neolithic. In M. Larsson, M. Parker Pearson (eds.), *From Stonehenge to the Baltic: Living with cultural diversity in the third millennium BC*. BAR IS 1692. Archaeopress. Oxford: 1–11.

Lidén K., Eriksson G., Nordqvist B., Götherström A., Bendixen E. and Lidén K. 2004. The wet and the wild followed by the dry and the tame-or did they occur at the same time? Diet in Mesolithic Neolithic southern Sweden. *Antiquity 78(299): 23–33*.

Linderholm A. 2011. The Genetics of the Neolithic Transition: New Light on Differences Between Hunter-Gatherers and Farmers in Southern Sweden. In *Human Bioarchaeology of the Transition to Agriculture*. John Wiley & Sons. Chichester: 385–402.

Malmstrom H., Gilbert M. T., Thomas M. G., Brandstrom M., Stora J., Molnar P., Andersen P. K., Bendixen C., Holmlund G., Gotherstrom A. and Willerslev E. 2009. Ancient DNA reveals lack of continuity between neolithic hunter-gatherers and contemporary Scandinavians. *Current Biology 19: 1758–1762*.

Manco L., Pires S., Lopes A. I., Figueiredo I., Albuquerque D., Alvarez M., Rocha J. and Abade A. 2013. Distribution of the – 13 910C*T polymorphism in the general population of Portugal and in subjects with gastrointestinal complaints associated with milk consumption. *Annals of Human Biology 40: 205–208*.

McCracken R. D. 1971. Lactase Deficiency: An Example of Dietary Evolution. *Current Anthropolog 12: 497–517*.

Melchior L., Lynnerup N., Siegismund H. R., Kivisild T. and Dissing J. 2010. Genetic diversity among ancient Nordic populations. *PLoS One 5: e11898*.

Nagy D., Bogacsi-Szabo E., Varkonyi A., Csanyi B., Czibula A., Bede O., Tari B. and Rasko I. 2009. Prevalence of adult-type hypolactasia as diagnosed with genetic and lactose hydrogen breath tests in Hungarians. *European Journal of Clinical Nutrition 63(7): 909–912*.

Nagy D., Tomory G., Csanyi B., Bogacsi-Szabo E., Czibula A., Priskin K., Bede O., Bartosiewicz L., Downes C. S. and Rasko I. 2011. Comparison of lactase persistence polymorphism in ancient and present-day Hungarian populations. *American Journal of Physical Anthropology 145: 262–269*.

Novembre J., Stephens M. 2008. Interpreting principal component analyses of spatial population genetic variation. *Nature Genetics 40: 646–649*.

Olds L. C., Ahn J. K. and Sibley E. 2011. 13 915*G DNA polymorphism associated with lactase persistence in Africa interacts with Oct–1. *Human Genetics 129: 111–113*.

Olds L. C., Sibley E. 2003. Lactase persistence DNA variant enhances lactase promoter activity in vitro: functional role as a cis regulatory element. *Human Molecular Genetics 12: 2333–2340*.

Pala M., Olivieri A. and 31 authors. 2012. Mitochondrial DNA signals of late glacial recolonization of Europe from near eastern refugia. *American Journal of Human Genetics 90: 915–924*.

Pavúk J. 2005. Typologische Geschichte der Linearbandkeramik. In J. Lüning, Chr. Frirdich and A. Zimmermann (eds.), *Die Bandkeramik im 21. Jahrhundert*. Symposium in der Abtei Brauweiler bei Köln vom 16.9.–19.9.2002. Internationale Archäologie 7. Vlg Marie Leidorf Rahden/ Westf.: 17–39.

Peng M. S., He J. D., Zhu C. L., Wu S. F., Jin J. Q. and Zhang Y. P. 2012. Lactase persistence may have an independent origin in Tibetan populations from Tibet, China. *Journal of Human Genetics 57: 394–397*.

Pinhasi R., Thomas M. G., Hofreiter M., Currat M. and Burger J. 2012. The genetic history of Europeans. *Trends in Genetics 28: 496–505*.

Plantinga T. S., Alonso S., Izagirre N., Hervella M., Fregel R., van der Meer J. W., Netea M. G. and de la Rua C. 2012. Low prevalence of lactase persistence in Neolithic South-West Europe. *European Journal of Human Genetics 20: 778–782*.

Reich C. M., Arnould J. P. 2007. Evolution of Pinnipedia lactation strategies: a potential role for alpha-lactalbumin? *Biology Letters 3: 546–549*.

Richards M. and 35 authors. 2000. Tracing European founder lineages in the Near Eastern mtDNA pool. *American Journal of Human Genetics 67: 1251–1276*.

Sahi T., Isokoski M., Jussila J., Launiala K. and Pyorala K. 1973. Recessive inheritance of adult-type lactose malabsorption. *Lancet 2: 823–826*.

Sahi T., Launiala K. 1977. More evidence for the recessive inheritance of selective adult type lactose malabsorption. *Gastroenterology 73: 231–232*.

Salque M., Bogucki P. I., Pyzel J., Sobkowiak-Tabaka I., Grygiel R., Szmyt M. and Evershed R. P. 2013. Earliest evidence for cheese making in the sixth millennium BC in northern Europe. *Nature 493: 522–525*.

Sanchez-Quinto F. and 12 authors. 2012. Genomic affinities of two 7,000-year-old Iberian hunter-gatherers. *Current Biology 22: 1494–1499*.

Scozzari R. and 15 authors. 2001. Human Y-chromosome variation in the western Mediterranean area: implications for the peopling of the region. *Human Immunology 62: 871–884*.

Semino O. and 16 authors. 2000. The genetic legacy of Paleolithic Homo sapiens sapiens in extant Europeans: a Y chromosome perspective. *Science 290: 1155–1159*.

Sherratt A. 1981. Plough and pastoralism: aspects of the Secondary Products Revolution. In I. Hodder, G. Isaac and N. Hammon (eds.), *Pattern of the Past: Studies in Honour of David Clarke*. Cambridge University Press. Cambridge: 261–305.

Simoni L., Calafell F., Pettener D., Bertranpetit J. and Barbujani G. 2000a. Geographic patterns of mtDNA diversity in Europe. *American Journal of Human Genetics 66: 262–278*.

2000b. Reconstruction of prehistory on the basis of genetic data. *American Journal of Human Genetics 66: 1177–1179*.

Simoons F. J. 1970. Primary adult lactose intolerance and the milking habit: a problem in biologic and cultural interrelations. II. A culture historical hypothesis. *American journal of digestive diseases 15: 695–710*.

Skoglund P., Malmstrom H., Raghavan M., Stora J., Hall P., Willerslev E., Gilbert M. T., Gotherstrom A. and Jakobsson M. 2012. Origins and genetic legacy of Neolithic farmers and hunter-gatherers in Europe. *Science 336: 466–469*.

Sun H. M., Qiao Y. D., Chen F., Xu L. D., Bai J. and Fu S. B. 2007. The lactase gene –13910T allele can not predict the lactase-persistence phenotype in north China. *Asia Pacific Journal of Clinical Nutrition 16: 598–601*.

Szilagyi A., Rivard J. and Shrier I. 2002. Diminished efficacy of colonic adaptation to lactulose occurs in patients with inflammatory bowel disease in remission. *Digestive Diseases and Sciences 47: 2811–2822*.

Thomas M. G., Kivisild T., Chikhi L. and Burger J. 2013. Europe and western Asia: genetics and population history. In I. Ness (ed.), *The Encyclopedia of Global Human Migration. Volume 1. Prehistory*. Blackwell Publishing Ltd./ John Wiley and Sons Ltd. Chicester: 146–156.

Tishkoff S. A., and 18 authors. 2007. Convergent adaptation of human lactase persistence in Africa and Europe. *Nature Genetics 39: 31–40*.

Torniainen S., Parker M. I., Holmberg V., Lahtela E., Dandara C. and Jarvela I. 2009. Screening of variants for lactase persistence/non-persistence in populations from South Africa and Ghana. *BMC Genetics 10: 31* doi:10.1186/1471-2156-10-31.

Torroni A. and 33 authors. 2001. A signal, from human mtDNA, of postglacial recolonization in Europe. *American Journal of Human Genetics 69: 844–852*.

Troelsen J. T. 2005. Adult-type hypolactasia and regulation of lactase expression. *Biochimica et Biophysica Acta 1723: 19–32*.

Troelsen J. T., Olsen J., Moller J. and Sjostrom H. 2003. An upstream polymorphism associated with lactase persistence has increased enhancer activity. *Gastroenterology 125: 1686–1694*.

Vigne J.-D. 2011. The origins of animal domestication and husbandry: a major change in the history of humanity and the biosphere. *Comptes rendus biologies 334: 171–181*.

2008. Zooarchaeological aspects of the Neolithic diet transition in the Near East and Europe, and their putative relationships with the Neolithic demographic transition. In J.-P. Bocquet-Appel, O. Bar-Yosef (eds.), *The Neolithic Demographic Transition and its Consequences*. Springer. New York: 179–205.

Vigne J.-D., Helmer D. 2007. Was milk a "secondary product" in the Old World Neolithisation process? Its role in the domestication of cattle, sheep and goats. *Anthropozoologica 42: 9–40*.

Vonk R. J., Priebe M. G., Koetse H. A., Stellaard F., Lenoir-Wijnkoop I., Antoine J. M., Zhong Y. and Huang C. Y. 2003. Lactose intolerance: analysis of underlying factors. *European Journal of Clinical Investigation 33: 70–75*.

Wang Y., Harvey C., Rousset M. and Swallow D. M. 1994. Expression of human intestinal mRNA transcripts during development: analysis by a semiquantitative RNA polymerase chain reaction method. *Pediatric Research 36: 514–521*.

Whittle A., Cummings V. 2007. *Going over: The Mesolithic-Neolithic transition in North-West Europe*. Oxford University Press. Oxford.

Zeder M. A. 2008. Domestication and early agriculture in the Mediterranean Basin: Origins, diffusion, and impact. *Proceedings of the National Academy of Sciences USA 105: 11597–11604*.

Zvelebil M., Zvelebil K. V. 1988. Agricultural transition and Indo-European dispersals. *Antiquity 62: 574–583*.