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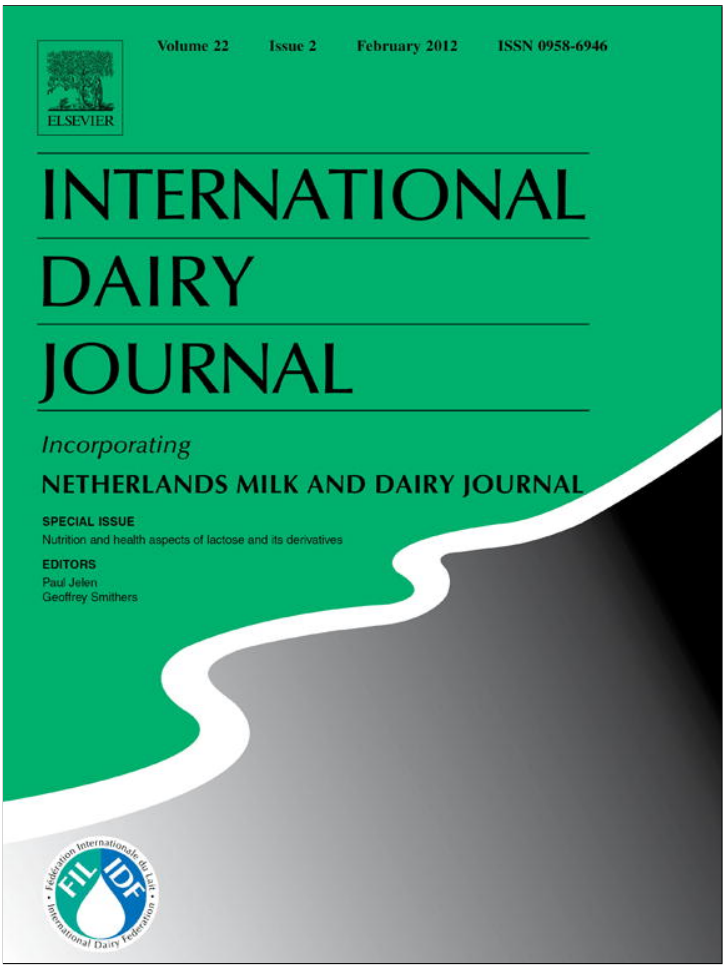

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International Dairy Journal 22 (2012) 88–97

Contents lists available at SciVerse ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Review

The evolution of lactase persistence in Europe. A synthesis of archaeological and genetic evidence

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ARTICLE INFO

Article history:
Received 14 February 2011
Received in revised form
7 October 2011
Accepted 9 October 2011

ABSTRACT

Lactase persistence, the ability to digest the milk sugar lactose in adulthood, is highly associated with a T allele situated 13,910 bp upstream from the actual lactase gene in Europeans. The frequency of this allele rose rapidly in Europe after transition from hunter–gatherer to agriculturalist lifestyles and the introduction of milkable domestic species from Anatolia some 8000 years ago. Here we first introduce the archaeological and historic background of early farming life in Europe, then summarize what is known of the physiological and genetic mechanisms of lactase persistence. Finally, we compile the evidence for a co-evolutionary process between dairying culture and lactase persistence. We describe the different hypotheses on how this allele spread over Europe and the main evolutionary forces shaping this process. We also summarize three different computer simulation approaches, which offer a means of developing a coherent and integrated understanding of the process of spread of lactase persistence and dairying.
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0958-6946/$ – see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.idairyj.2011.10.010

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1. Introduction

It has been known at least since Roman times that individuals vary in their ability to digest milk. The reason for this is that milk contains the disaccharide sugar lactose, which can only be broken down into its monosaccharide constituents, glucose and galactose, when the enzyme lactase is present in the duodenum. In most mammals, including most humans, the production of lactase is down-regulated shortly after the weaning period is over. Consumption of milk for such individuals results in lactose digestion by colonic bacteria, leading to the production of fatty acids and various gasses, especially hydrogen. In addition, the presence of lactose in the colon has an osmotic effect, drawing water in from the blood. The outcomes can include diarrhoea, cramps, bloating and chronic flatulence. Symptoms like this are usually referred to as lactose intolerance although their severity is variable depending on the quantities of lactose consumed, colonic flora and possibly additional factors like other components of the diet. About 35% of people in the world continue to produce lactase throughout adulthood and thus are able to digest the sugar in milk without discomfort (Ingram, Mulcare, Itan, Thomas, & Swallow, 2009); a trait known as lactase persistence (LP). Genetic data clearly indicate that lactase persistence has been subject to very strong positive natural selection in the last 10 thousand years or so – a time period that brackets the domestication of dairying animals and of milk exploitation.

Milk became available for adult consumption only after sheep, goat and cattle were domesticated in south-eastern Anatolia and the Near East at the onset of the Neolithic some 10,500 years ago. The role of milk production in the early Neolithic had long been disregarded, but in the last ten years new analytical techniques have demonstrated that milk was used not long after the beginnings of domestication. Some authors even propose milk availability as the reason why Neolithic populations began to domesticate wild animals (Vigne & Helmer 2008). However, it is very unlikely that our Neolithic ancestors were lactase persistent, and so able to consume fresh milk in significant amounts without suffering the associated consequences (Burger, Kirchner, Bramanti, Haak, & Thomas 2007; Malmström et al., 2010). As some early Neolithic populations were producing milk but could probably not digest it, they were most likely processing it to produce cheese, yoghurt, butter and other products with reduced lactose content that are more easily digestible.

The biological evolution of LP is thus intimately entwined with the cultural evolution of dairying. LP could only have been selectively favoured among people with a supply of fresh milk, and dairying would have been more beneficial to lactase persistent than lactase non-persistent populations. This is probably the best known, best supported and most often cited example of gene-culture co-evolution. It is not possible to understand one without the other. For these reasons it has become increasingly important to understand the archaeology of dairying. In this article we will try to summarize evidence from archaeology, molecular biology and evolutionary genetics to reconstruct the historic and demographic processes that led to the rapid spread of LP over the last 10,000 years.

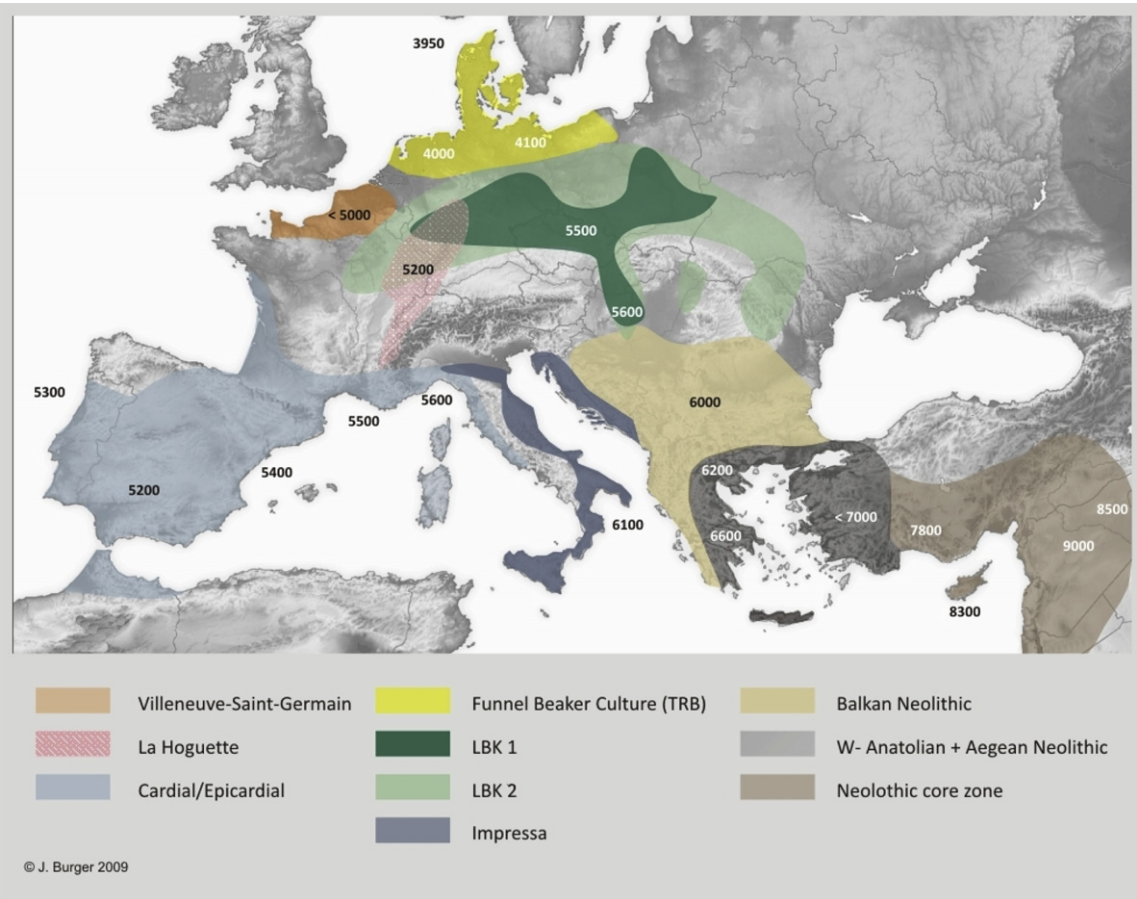

Fig. 1. Chronological spread of the Neolithic (after Burger & Thomas, 2011).

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2. The Neolithic transition in Europe

Neolithisation is a cultural process defining the transition from a Palaeolithic–Mesolithic semi-nomadic lifestyle with an economy based on hunting and gathering, to a Neolithic sedentary culture, in which agriculture and domestic animal exploitation become the dominant subsistence strategies. The Neolithic transition was a long and complex process of acquisition of social behaviours connected to sedentary settlements, development of new economic strategies (such as animal and plant domestication) and technical innovations (e.g., pottery, polished stone tools). Management of domesticated plants and animals not only required important technological innovations and skills, but also entailed various changes to the domesticated species themselves. Some of these changes are environmental, due to selection of plants and animals that lead to the development of domestic forms, which contributed to shaping farming-like landscapes and in some cases even replaced their wild progenitors (e.g., Lüning 2000). Other changes involved the general organization of the society, and resulted in subdivision of labour, creating the basis for personal versus communal property. These changes illustrate the consequences of the replacement of a nomadic Palaeolithic hunter–gatherer culture by a sedentary one, leading to an increasingly structured society (Bar-Yosef, 2001).

The beginnings of the Neolithic transition date back to about 12,000 years before present (BP). It first developed in a core zone in the Near East and Anatolia, from where it spread to the Middle East, the Caucasus, Europe, and Africa (Fig. 1). The development of Neolithic culture in these regions is also called "Neolithisation", but the mechanisms of spread are distinct from the initial process of innovation in the core region. In Europe, for instance, archaeological evidence indicates a long and progressive acquisition of techniques possibly mediated by immigrants or pioneers and only in part influenced by indigenous hunter–gatherer traditions (Gronenborn, 1997). Two different scenarios have been proposed for explaining this transition in Europe. The demic diffusion model assumes that the diffusion of Neolithic culture into Europe from the Near East was mediated by the migration of a significant number of farmers without substantial mixing with local Palaeo-Mesolithic populations (Ammerman & Cavalli-Sforza, 1984). On the contrary, the cultural diffusion model posits a Neolithic transition mediated mainly through the transmission of agricultural skills and techniques, without large movements of people (Zvelebil & Zvelebil, 1988). Latest theories in archaeology suggest that the dynamics of the spread of agriculture over Europe was more complex, with a succession of migration phases and local admixture (Lübke, Lüth, & Terberger, 2009; Zvelebil, 2004).

Many attempts have been made to test those models, without finding unequivocal answers. Ancient DNA data have recently shown clear evidence for discontinuity between the last hunter–gatherers and early farming populations in central Europe (the so-called Linearbandkeramik culture, LBK, around 7500–7000 BP), supporting a demic diffusion model at least for this region (Bramanti et al., 2009). However, admixture with local hunter–gatherers is likely to have happened later (Burger, 2010). The question is whether this immigration of farmers was accompanied by the diffusion of a dairying culture into the new area. To answer this, we first have to look at the arrival of domesticates, an undeniable prerequisite for dairying.

3. Spread of domesticates from the Near East to Europe

Animal domestication started in a region between the Zagros and the Taurus mountains, possibly in the Middle Euphrates valley around 11,000 BP for goat and sheep, and around 10,500 for cattle and pig (Helmer, Gourichon, Monchot, Peters, & Saña Segui, 2005; Peters, Helmer, Von Den Driesch, & Saña-Segui, 1999; Peters, Von Den Driech, & Helmer, 2005; Vigne, Carrère, & Guilaine, 2003). By 9000 BP, domesticates were already distributed in a large part of the Near and Middle East and Anatolia (Guilaine, Briois, Vigne, & Carrere, 2000; Vigne & Buitenhuis, 1999), expanding to Western Anatolia after 9000 BP, and to Greece and the Balkan region after 8400 BP (Guilaine, 2003; de Keroualin, 2003; Perlès, 2001). From here, the diffusion of domesticates took two different routes, a costal route through the Aegean, Adriatic and Tyrennian sea, and a continental route along the Danube through the Balkans and into Central Europe (Tresset & Vigne, 2007).

Oriental mufflon (*Ovis orientalis*) and bezoar goat (*Capra aegagrus*) are phylogenetically the closest wild relatives of domesticated sheep (*Ovis aries*) and goat (*Capra hircus*), respectively. Neither of these wild species would have been present in Europe prior to the Neolithic, and their natural distribution is restricted in a region that encompasses the Neolithic core zone. Therefore, both domesticate sheep and goat could only be the result of domestication of *O. orientalis* and *C. aegagrus*, which occurred in the Near East and consequently spread throughout Europe. For cattle the situation is different, because aurochs (*Bos primigenius*), the wild progenitor of taurine cattle (*Bos taurus*), was dispersed throughout continental Eurasia. However, genetic studies of both wild and domestic cattle indicate that the domestic form was also imported from the Near East, and that in central Europe little or no interbreeding occurred (Bollongino, Edwards, Alt, Burger, Bradley, 2006; Bollongino, Elsner, Vigne, & Burger, 2008; Edwards et al., 2007; Troy et al., 2001). Thus, around 8400 BP three forms of milkable animals appeared in the south and the southeast of Europe: cattle, goat, and sheep. All of them were previously domesticated in Anatolia or the Near East and subsequently transported into Europe.

4. The archaeology of dairying

4.1. Milk – a "secondary product"?

Domestication allowed humans to utilize many different animal products, meat being only the most obvious among these. The so-called "Secondary Product Revolution" model (Sherrat, 1981, 1983) proposes that during the early Neolithic the economy was based on "primary" products, the ones that can be extracted only after the death of an animal (meat, hide, bone, horn, etc.). Then, during the Calcholithic and Bronze Age, new animal management strategies were developed. It was proposed that production of "secondary" products, i.e., those that are harvested during the animal's lifetime (milk, wool, labour, dung), lead to an economical and political revolution. Milking provided a new source of energy without slaughtering the animal, while the use of animal power would enable the intensification of agricultural production and increased the possibility of transport, trade, and personal mobility. New regions were colonized with the help of secondary products and, as shown by settlement organization, the society then became more complex and hierarchical (Greenfield, 1988, 2005; Sherrat, 1981).

However, the "secondary product revolution" hypothesis has been widely criticized (for a review see Greenfield, 2010; Vigne & Helmer, 2007). In the last 10 years research from different fields provided new insights on the appearance of these products, with new dates that partially invalidate this hypothesis (e.g., Craig et al., 2005; Evershed et al., 2008; Greenfield, 2010). Most of the new evidence concerning the "secondary product" milk comes from advances in archaeozoological methods. This deserves a closer look.

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4.2. Evidence for dairying from archaeozoology and fat residues in pottery

Indications of milk production can be provided by the archaeozoological analysis of skeletal assemblages. The various ways animals are exploited (meat, milk, wool production, etc.) influence breeding practices and slaughter profiles. In archaeological bone assemblages this can be detected as distinct patterns of distribution of both animal sex and age at death. Thus, by analyzing age and sex composition of an archaeozoological assemblage it is – in theory – possible to identify the exploitation strategy (Payne, 1973). Theoretically, the ideal dairying profile would require the culling of most animals younger than two months to allow humans to use most of the milk. An optimal meat strategy, on the other side, involves the harvesting of most animals after one to three years, when they achieve their maximum weight (Mlekuz, 2006). It is clear that varying or mixed production strategies may produce similar harvest profiles, but it has been demonstrated that these tend to obscure milk patterns, rather than to create them artificially (Halstead, 1998). One of the main problems with this approach is the determination of age at death. It could be assigned on the basis of the degree of bone development, but such an approach lacks accuracy. A better criterion is the eruption of the teeth and replacement of milk teeth by the definitive ones, if available (Ducos, 1968). On the basis of slaughtering age profiles Vigne (2008) and Vigne and Helmer (2007) have shown that the exploitation of sheep, goat and cattle in the Middle East and in Mediterranean Europe is consistent with milk production from the early Neolithic onwards.

Another approach that led to a critical re-evaluation of the timing and nature of prehistoric milk usage is the archaeometric analysis of organic residues in pottery. Archaeological ceramics often contain many different kinds of organic residues, among them fats. It is possible to identify degraded animal fats on the basis of their chemical and stable isotope composition. The difficulty is to differentiate fresh milk fats from adipose fats because the chemical distinction between them is partly erased by diagenetic alterations since death. However, it has been shown that adipose and milk fat metabolisms lead to significant differences in $\delta^{13}C$ isotope ratio values of various fatty acids, giving a criterion for detecting milk fat residues in pottery (Dudd & Evershed, 1998). Using this approach, the exploitation of milk in the early Neolithic has been demonstrated to have occurred around 8000 years ago in Northwestern Anatolia and Thrace (Evershed et al., 2008), around 7000 years ago in the Carpathian Basin (Craig et al., 2005) and few hundred years later in Britain (Copley et al., 2003).

These data from Anatolian sites also provide us with complementary information. While experiments on modern ceramics have shown that burial processes rapidly destroyed raw milk fats, such lipids have been detected in ceramics dated from thousands of years ago. The most likely explanation for this is that the detected dairy fat residues come from fermented milk products such as yoghurt and cheese and that their detection indicates not only dairying, but also milk processing. This processing is likely to have offered a number of advantages, including: (1) providing a means of storing milk products such as cheese, making them available in times of low milk production; (2) providing better transportation possibilities when people leave their settlement seasonally, or for transhumance; (3) reducing or eliminating lactose, thereby rendering milk products digestible by lactase non-persistent individuals. The last advantage is likely to have been of particular importance since genetic data are making it increasingly clear that these early dairying populations were mostly, if not entirely lactose intolerant.

5. Lactase persistence

5.1. Milk digestion

Lactose, the main milk sugar, is a disaccharide composed of one molecule of glucose and one molecule of galactose. It can be hydrolyzed into its two constituent monosaccharides by an enzyme called lactase (lactase phlorizin hydrolase, LCT), produced mostly in the small intestine of mammals. Lactase shows a tissue specific expression in the small intestine, with a differential expression along its longitudinal axis (with higher expression in the jejunum). Within the jejunum, lactase expression also varies at the cellular level, as it can be detected in differentiated enterocytes located at the crypt/villus junction (Troelsen, 2005). However, in both rat and human, minor lactase mRNA expression can be observed in the colon during the postnatal period (Freund et al., 1990; Wang, Harvey, Rousset, & Swallow, 1994).

Humans show high lactase expression at birth (Wang et al., 1998), while in some other mammals, as rodents, the level of expression is relatively low just after birth and reaches its maximum two to three days later, when the intestine is mature (Troelsen, 2005). In all mammals, high lactase expression continues until the weaning period is over. At that time, the diet changes from one based exclusively on milk to a more complex one involving different sources. This is when lactase expression is usually down-regulated although in humans the age at which this occurs is variable. About 35% of people worldwide are lactase persistent and – with the exception of unusual challenges such as gut trauma – produce lactase throughout adulthood (Swagerty, Walling, & Klein, 2002). In lactase non-persistent individuals, small intestine lactase activity is usually insufficient to hydrolyze all ingested lactose. Consequently, some lactose enters the colon where colonic microbiota first convert it into glucose and then ferment it, producing short chain fatty acids and gases. In combination with the osmotic effects of having undigested lactose in the colon, this can cause the above mentioned unpleasant symptoms (Ingram et al., 2009).

Digestion of lactose by micro-organisms in the small intestine seems not to be the only factor influencing lactose intolerance. For example, lactose maldigesters with a similar oro-caecal transit time and degree of lactose digestion in the small intestine develop symptoms of different severity (Vonk et al., 2003). Colonic fermentation can aggravate or alleviate the symptoms of lactose intolerance. This depends on the balance between the ability of colonic microbiota to ferment lactose and the ability of the colon to remove the metabolites of this fermentation (He et al., 2008). It has been shown that daily lactose ingestion can lead to colonic adaptation and reduced symptoms, via increasing the capacity to ferment lactose and inducing a metabolic shift, reducing production of hydrogen by bacteria (Szilagyi, Rivard, & Shrier, 2002).

5.2. Distribution of lactase persistence phenotype

The lactase non-persistence phenotype is common in adult humans (65%, Ingram et al., 2009; Itan, Jones, Ingram, Swallow, & Thomas, 2010), and its geographic distribution is not uniform since lactase persistence shows a correlation with a history of pastoralism and/or dairying. LP is prevalent in Europe, with the highest frequencies in the northwest of the continent (0.89–0.96 in the British Isles and Scandinavia), showing a decreasing cline towards the southeast where its frequency can be as low as 0.15 around the eastern Mediterranean (Ingram et al., 2009; Itan et al., 2010) (Fig. 2). A similar cline is observed in India, with higher frequencies of the trait in the north (0.63) and lower frequencies in the south (between 0.2 and 0.1; Itan et al., 2010; Swallow & Hollox,

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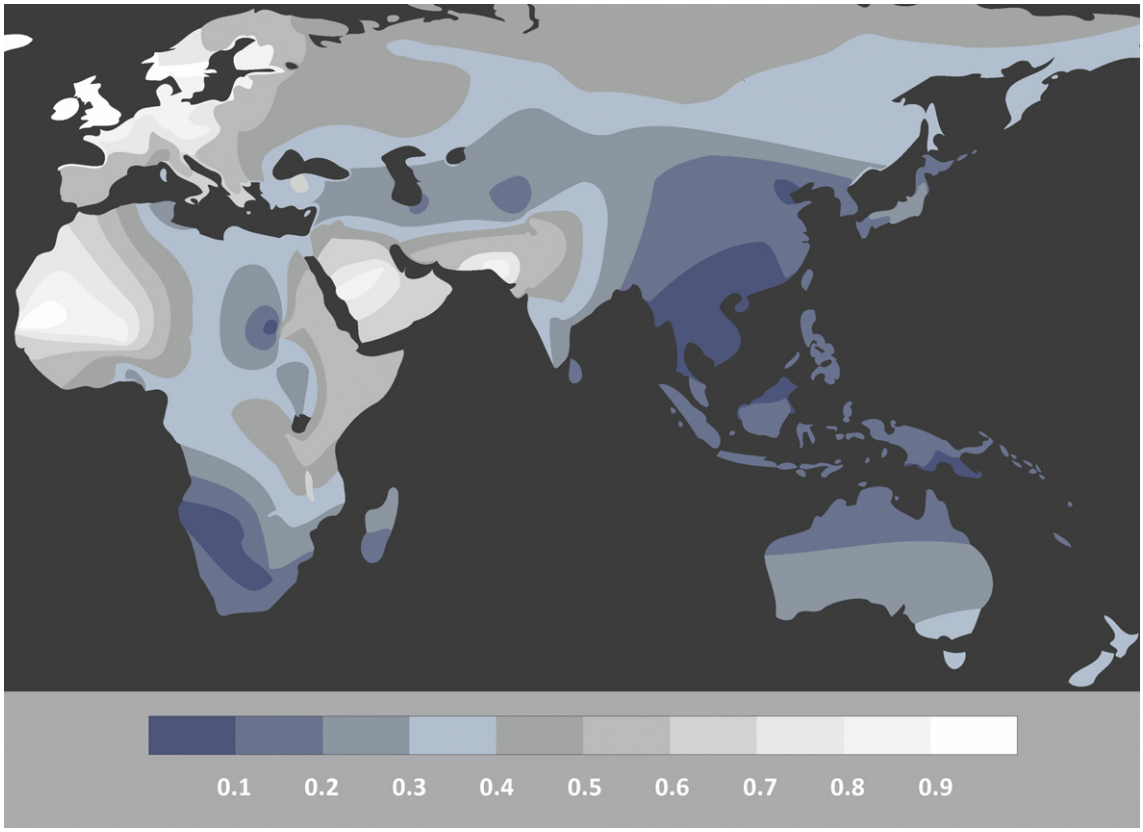

Fig. 2. Worldwide frequencies of lactase persistence phenotype (after Itan et al., 2010).

2000). It is rare in Native Americans and in eastern Asians (Itan et al., 2010).

In Africa, LP distribution is very patchy (Swallow, 2003) as interspersed pastoralist populations tend to present high frequencies of the trait, whereas neighbouring non-pastoralist groups typically have much lower frequencies. In Rwanda, for example, the proportion of lactase persistent individuals varies from 0.02 in Bashi to 0.92 in Tutzi (Cox & Elliott, 1974; Ingram et al., 2009). This pattern is also seen in Middle East, where Bedouin and non-Bedouin populations from the same geographic area show a significant difference in lactose digestion capacity. This has been observed in both Jordan and Saudi Arabia, with a LP frequency varying respectively from 0.76 to 0.23 and from 0.86 to 0.22 (Ingram et al., 2009).

5.3. Molecular causes of lactase persistence

LP is an autosomal dominant trait (Enattah et al., 2002). Lactase production is genetically determined by a single gene, LCT, located on chromosome 2, which is 49,336 base pairs (bp) in length (NCBI Reference Sequence NG_008104). An analysis of the whole gene and its promoter region showed that it contained many polymorphisms organized into a small number of haplotypes (Hollox et al., 2001). However, while haplotypes that clearly associated with LP were found at that time, the causative polymorphism, or polymorphisms, remained elusive. Later, Poulter et al. (2003) identified a region of very high linkage disequilibrium, with many variable sites showing a high level of association with LP. This region is located upstream of the lactase gene transcription initiation site (Poulter et al., 2003). Another study on Finnish individuals found a single nucleotide polymorphism in the same region (−13,910 C/T) which, at the time, was reported to associate completely with LP (Enattah et al., 2002). This polymorphism was in what appeared to be a cis-acting regulatory element located in the 13th intron of a neighbouring gene, MCM6 (which is involved in cell cycle regulation). It was subsequently shown in functional studies that the T variant is a more effective enhancer of LCT promoter activity than the −13,910*C variant (Olds & Sibley, 2003; Troelsen, Olsen, Møller, & Sjöström, 2003).

While in Europe the −13,910*T allele appears to explain the LP phenotype distribution very well, in the Near and Middle East, and in Africa the situation is not so clear. Mulcare et al. (2004) showed that this allele could not explain the distribution of LP in many African populations with appreciable frequencies of the trait. Subsequent studies identified different alleles, on different haplotypic backgrounds, that associated with LP in those regions (Enattah et al., 2008; Ingram et al., 2007; Tishkoff et al., 2007). Nonetheless, comparisons of LP phenotype frequencies with the distribution of currently known LP-associating alleles indicate that our knowledge of the genetic causes of LP is incomplete in some regions (e.g., Western Africa, Eastern Africa). It is thus likely that further genetic variants remain to be found (Itan et al., 2010).

5.4. Palaeogenetics of lactase persistence

Since the ancestral state of LP is non-persistence, and since milk exploitation is unlikely to have started before the Neolithic, an interesting question is whether early Neolithic populations were lactase persistent or not. Although we cannot know the exact phenotype frequency in past populations, it can be estimated by examining DNA from the skeletons of individuals living at the time. Obtaining reliable ancient DNA data has its own set of challenges, among them avoiding contamination from modern sources. However, a few ancient DNA studies have managed to produce

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reliable data on the −13,910 C/T polymorphism. One Mesolithic and eight Neolithic European skeletons were examined by Burger et al. (2007) and found not to carry the −13,910*T allele, suggesting that LP frequency was significantly lower in early Neolithic Europeans than it is today, and may have been zero. Analysis of 10 skeletons from a Middle Neolithic hunter–gathering population in Scandinavia also indicated a large difference in LP frequency between ancient and modern populations; they found nine individuals who were homozygous for the −13,910*C allele and one heterozygous individual (Malmström et al., 2010).

5.5. Selection of lactase persistence

While palaeogenetic studies can allow us to estimate LP-causing allele frequencies at various points in space and time, and have indicated that LP was rare or absent in early Neolithic central Europe, they cannot tell us directly how old these alleles are. However, using long-range haplotype conservation (Bersaglieri et al., 2004) and variation in closely linked microsatellites (Coelho et al., 2005), the −13,910*T variant has been estimated to be between 2188 and 20,650 years old and between 7450 and 12,300 years old, respectively. It is interesting to note that similar but slightly younger age estimates have been obtained for one of the major African LP variants, −14,010*C (Tishkoff et al., 2007). Such age estimates for −13,910*T bracket the Neolithic and are consistent with palaeogenetic data, but indicate a rapid rise in frequency in the intervening time; modern day LP frequencies in the same areas reach 0.60 in Central Europe, 0.92 in Finland, 0.97 in the United Kingdom and 0.98 in Denmark (Ingram et al., 2009). This dramatic change of LP frequency in a relatively short time span cannot be explained by genetic drift alone; very strong natural selection needs to be invoked.

There are many possible reasons why the ability to digest milk may have been positively selected (for a fuller discussion on the positive effects of milk drinking in prehistory see Gerbault et al., 2011) but does one of them possess sufficient explanatory power for the enormous LP frequency change we observe in the last 8,000 years?

As stated above, there is a strong correlation between lactose digestion and milking practices in many geographical regions worldwide. This can be explained in two different ways: milk drinking could have been adopted in populations who could tolerate it by being genetically pre-adapted (reverse-cause argument, McCracken, 1971), or LP-associated alleles could have been positively selected in dairying populations (the culture-historical hypothesis) (McCracken, 1971; Simoons, 1970). The first hypothesis is not supported by the available ancient DNA data, and does not provide a straightforward explanation for the original differentiation in LP frequencies between populations. It is also difficult to reconcile with the independent, convergent evolution of LP in different parts of the world, as evidenced by the existence of multiple genetic causes in different regions. However, the second hypothesis suggests that the modern pattern of variation could be the result of positive selection on persistent individuals, who would have had access to dairy products. This second hypothesis is indeed supported by archaeological and palaeogenetic data because, as already discussed, the first evidence of dairying practices dates back to the early Neolithic (Copley et al., 2003; Craig et al., 2005; Evershed et al., 2008; Greenfield, 2010; Vigne & Helmer, 2007), a time when LP-associated allele frequencies were very low or zero (Burger et al., 2007; Malmström et al., 2010).

A closer look at Europe shows that LP frequencies correlate with latitude, and this evidence was used by Flatz and Rotthauwe (1973) to formulate the Calcium Assimilation Hypothesis. Milk contains many different nutrients, including large amounts of calcium and very small amounts of vitamin D. In the human body, vitamin D is normally produced photochemically in the skin through the action of sunlight, or assimilated from a diet rich in marine food. Since vitamin D regulates calcium absorption, a lack of sunlight exposure, if not substituted by a vitamin D rich diet, could lead to osteological malformations such as rickets. Dairying populations would be able to supplement their vitamin D and calcium intake and so avoid the potential problems associated with the low vitamin D, cereal-rich diet at northern latitudes. Isotope analysis, carried out on skeletal populations living on the coasts of Sweden could give an indirect support for this hypothesis. A switch from an almost exclusively marine diet in Mesolithic to a mixed marine-terrestrial in Middle Neolithic individuals was shown, when the first traces of LP have been found (Malmström et al., 2010), and eventually to a mainly terrestrial diet during the Late Neolithic period (Eriksson et al., 2008; Lidén & Eriksson, 2007; Lidén; Eriksson, Nordqvist, Gotherstrom, & Bendixen, 2004). Furthermore osteological analyses revealed a reduction in general health status following the switch to farming, thereby supporting the calcium assimilation hypothesis (Cohen, 2008; Eshed, Gopher, Pinhasi, & Hershkovitz, 2010; Hershkovitz & Gopher, 2008; Siegmund, 2010; Larsen, 1995; personal communication from Christina Papageorgopoulou, Johannes Gutenberg University, Mainz, Germany).

A third hypothesis has been proposed, suggesting that LP would give a big advantage in arid environments by providing a good source of uncontaminated fluid, while maldigestion symptoms such as diarrhoea would give major problems to non-persistent individuals, and could even cause death (Cook, 1978; Cook & al-Torki, 1975). However, the European temperate climate makes this hypothesis less likely to explain the continental distribution of milk digesters.

An attempt to test these three hypotheses was made by Holden and Mace (1997). They used a comparative phylogenetic method to test the three hypotheses presented above: the calcium assimilation, the adaptation to arid environments and the gene-culture coevolution hypotheses. The authors created three phylogenetic trees linking many populations worldwide on the basis of languages and genetic variation (F_{ST}, Gene Diversity). Then they tested for correlation between LP and the following quantitative traits: pastoralism, solar radiation/dry months per year, average rainfall. The aim of the work was to see if the increase of frequency of LP is correlated with milking behaviour or not. To do this they compared a model of dependent change (correlating the two variables) with a model of independent change (not correlating the variables). The analysis showed that pastoralism explains the greater amount of LP frequency variation worldwide. Neither solar radiation, nor aridity gave the same result. The study suggests that the evolution of LP is strongly associated with the presence of pastoralism and furthermore that pastoralism is always adopted before the acquisition of the ability to digest milk.

While the signatures of positive selection on LP are widely accepted, it is still not clear what kind of selective forces are responsible for the high frequencies of LP in many populations today. Since the practice of milk fermentation (to make yoghurt, sour milk, etc.) as well as cheese making reduces lactose content, allowing non-persistent individuals to benefit from milk products (Hammer, Hammer, & Kletter, 1998), selection should act only in fresh milk drinking populations. Moreover, the consumption of fresh milk in small quantities is another mean by which lactose non-persistent individuals avoid the unpleasant symptoms of lactose maldigestion. Those practices nowadays allow some groups with low LP phenotype frequencies (Mongols, Herero, Nuer, Dinka) to include fresh milk in their diet (Swallow, 2003). If non-persistent individuals have access to many of the same nutritional benefits by fermenting the milk, it becomes more difficult to explain why LP shows such high selection coefficients. Certain demographic processes such as allele surfing (Edmonds, Lillie, & Cavalli-Sforza,

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2004; Klopfstein, Currat, & Excoffier, 2006; Travis et al., 2007) can cause genomic regions to mimic the signatures for selection seen in the LCT gene. However, if such effects had occurred we would expect to see such signatures through the genome in the same populations – which is not observed.

It is certainly important to take into account the possible social effects related with the ownership of domestic animals, especially cattle. From a socio-historical point of view it has been proposed that, since in many human cultures livestock are kept by a social elite, milk drinking could be restricted to a small part of the population, as a result of social and economic stratification (Burger, 2010; Gillis, 2003; Simoons, 1970). If milk drinking behaviour and a LP-associated allele were both present in such an elite, the effects of natural selection could be amplified by a different reproductive behaviour between social strata (Heyer, Sibert, & Austerlitz, 2005).

5.6. Modelling the origin of lactase persistence in Europe

Allele frequency fluctuations in a population result from the interaction of several processes, including mutation, selection, the mating system of the population (i.e., heterogamy, homogamy) and genetic drift shaped by demographic history. A number of these processes can, in turn, be shaped by social structure and culture. We have already presented a variety of evidence underlining the role of selection in shaping the modern pattern of LP distribution in Europe, but computer simulations are the best current approach to test more complex models integrating a constellation of factors in a more realistic framework.

One of the first formal models of gene-culture co-evolution that was applied to the evolution of LP was proposed by Aoki (1986). This model was designed to investigate if the incomplete correlation between lactase persistence and milking habits could be expected under a stochastic process of gene-culture co-evolution. He assumed 4 different gene-cultural phenotypes: milk-drinker lactase persistent, milk-drinker non-persistent, milk-non-drinker persistent and milk-non-drinker non-persistent, considering only the first two as being positively selected. A starting population size of 100 individuals was set. The adults mate at random, producing an infinite number of offspring. The probability of transmission of milk drinking habits was different on the basis of the lactase persistent or lactase non-persistent status of the parents. He then sampled 100 individuals randomly chosen from the offspring generation. At the end of the whole process Aoki (1986) calculated the correlation between the probability of fixation of LP and milk drinking behaviour, to determine if there was any difference in fixation rates between both cultural and genetic traits. He also examined the time necessary for LP to be fixed, to test if the time span necessary to reproduce the modern LP frequencies was in accordance with the onset of domestication in Europe. The results demonstrated that under a gene-culture co-evolutionary model an incomplete correlation between LP frequencies and milk drinking habits is expected due to the stochastic nature of the process. This finding has indeed a biological meaning, as some milk-drinkers may not be lactase persistent, while some milk-non-drinkers may be lactase persistent.

Gerbault, Moret, Currat, and Sanchez-Mazas (2009) simulated the evolution of the frequency of a dominant allele associated with LP from the beginning of the Neolithic (10,000 years ago) in Near Eastern and European populations. They tested four different scenarios, comparing the demic diffusion and cultural diffusion models for the dissemination of farming over Europe, with constant selection on the LP-associated allele or with a selection varying with latitude. The latter was used to estimate support for the calcium assimilation hypothesis. They also explored two additional scenarios of demic versus cultural diffusion in which selection was higher in populations belonging to the early Neolithic Linearbandkeramik culture (LBK) area in Central Europe. The temperate climate in Central and Northern Europe may allow milk to stay fresh longer than in the Mediterranean basin, and thus it is possible that

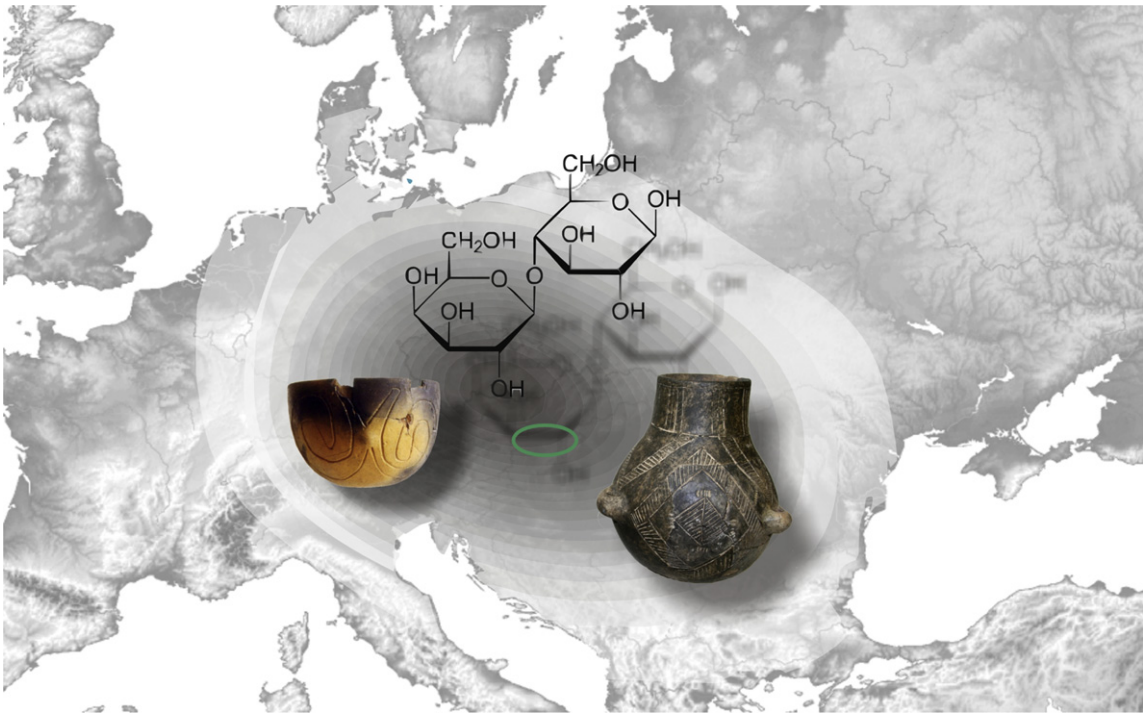

Fig. 3. Best estimates for the region where the lactase persistence associated allele was first subjected to selection (after Itan et al., 2009). It overlaps well with the region where the linear pottery culture (LBK) developed (green circle). The two pots represent typical containers of the LBK (photo courtesy of Sabine Schade-Lindig, Wiesbaden) and the lactose molecule is illustrated.

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selection has been stronger in LBK-related populations or Neolithic populations following the LBK. These 6 scenarios were finally compared using an Approximate Bayesian Computation (ABC) approach (Beaumont, Zhang, & Balding, 2002) based on Euclidean distances between observed and simulated allele frequencies, using a weighted multiple linear regression. First of all, any model with constant selection over the whole continent was rejected, because in every scenario genetic drift alone was able to explain the LP-associated allele frequencies in southern Europe, while to get northern European population frequencies, higher selection coefficients were required. Therefore, they found that modern diversity patterns are better explained under the demic diffusion model associated with the calcium assimilation hypothesis.

Itan et al. (2009) reached different conclusions. In their study, they used a spatial framework to simulate the spread of dairying associated with the diffusion of the −13,910*T allele in three interacting cultural groups (hunter–gatherers, non-dairying farmers and dairying farmers). They initiated a continent that was already settled by hunter–gatherers then simulated (starting 9,000 years ago) both farming populations expanding from Anatolia into Europe. Interactions between these 3 populations, as well as the expansion of farmers, were modelled through four kinds of migratory process: interdemic gene flow among cultural groups, intrademic gene flow between cultural groups, sporadic long distance migration among cultural groups, and cultural diffusion of subsistence practices. In addition, they modelled other processes such as natural selection acting on the LP allele. The authors considered the LP allele being selected only in dairying farmers, as they were the sole group to exploit milk in the model. They then traced the expected proportion of genetic ancestry from the geographic region where −13,910*T/dairying co-evolution originated. The best simulations were first identified by comparing simulated and observed data on the −13,910*T allele frequency in 12 locations and on the arrival date of farming in 11 of those 12 locations, using an ABC approach (Beaumont et al., 2002). Then, and this is one of the advantages of ABC methods, posterior estimates of unknown parameters, such as migration rates and selection intensity were made from these best simulations. Amongst these parameters, the east–west and north–south coordinates of where the −13,910*T allele first underwent selection and the time when that selection started were estimated. The best fit to the observed data was obtained for simulations in which selection for that allele originated 6256 to 8683 years BP in a region between the north Balkans and central Europe. As shown in Fig. 3 both this period and this region match well with the early dissemination of the LBK culture (Pavuk, 2005). Furthermore, the authors showed that a latitudinal effect on selection was unnecessary to explain the high frequencies found in northern Europe, as the simulated frequencies they got tended to be higher than the observed ones without additional effect on selection.

The discrepancies between these latter two studies come from the different ways allele diffusions associated with farming spread was modelled. In Gerbault et al. (2009), the LBK scenario simulated an increase of selection intensity restricted to populations assumed to be descendants of LBK populations. Therefore, non-LBK populations in Northern Europe were not considered to benefit from that increase of selection intensity, and allele frequencies as high as observed in those populations could not be obtained from simulations under that scenario. It should be noted that the frequency of the LP-associated allele was considered to be shaped by population growth, genetic drift and natural selection, but this study did not take into account gene flow between neighbouring populations. This was because: (1) this model was not fully spatially explicit, and (2) designating 'neighbouring' status to the populations considered in this study would have been subjective, and based on controversial archaeological data. Thus, the rejection of the LBK scenario from Gerbault et al. (2009) simply shows that higher selection coefficient in LBK-related populations are insufficient on their own to account for the allele frequencies observed over the European continent. This does not contradict the findings of Itan et al. (2009) about the time and location of origin of selection of the −13,910*T allele. Furthermore, the way the calcium assimilation hypothesis was tested in Gerbault et al. (2009) allows for the formulation of an alternative hypothesis that would postulate that selection intensity for LP was neither constant through time, nor through space. It should be noted however that Itan et al. (2009) did not model spatio-temporal variation in selection intensity throughout the continent, so this hypothesis was not formally rejected.

6. Conclusion

From the analysis of LP-associated genetic variants it is clear that the evolution of the ability to consume appreciable amounts of fresh milk has been a complex process in which many physiological, genetic, social but mainly evolutionary and demographic factors need to be considered. Computer simulations in conjunction with modern statistical methods have provided a powerful means for investigating and integrating the role of such factors, and have provided us with inferences on this co-evolutionary process that, to a first order of approximation, fit well with archaeological data. However, all models are simplifications and as new data become available from genetics, including ancient DNA, and archaeology, those models will require refinement.

LP provides a striking example of recent, and may be even ongoing evolution in humans, and of how genes and culture can interact in shaping our biology. It also highlights how important it is to integrate advances of knowledge in a range of distinct scientific disciplines. By incorporating several sources of evidence, we are more likely to arrive at a coherent understanding of the evolution of LP. Similarly, the study of when dairying started may bring insights on how agriculture spread from the Neolithic core zone. This is non-trivial when one considers how our societies have changed since the beginnings of the Neolithic. In this respect the domestication of milkable animals and the invention of agriculture together with rise of LP is a paradigm of successful niche construction of the human species.

Acknowledgement

M. L. and P. G. are funded from an EU Marie Curie FP7 Framework Programme grant (LeCHE, grant ref. 215362-2).

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