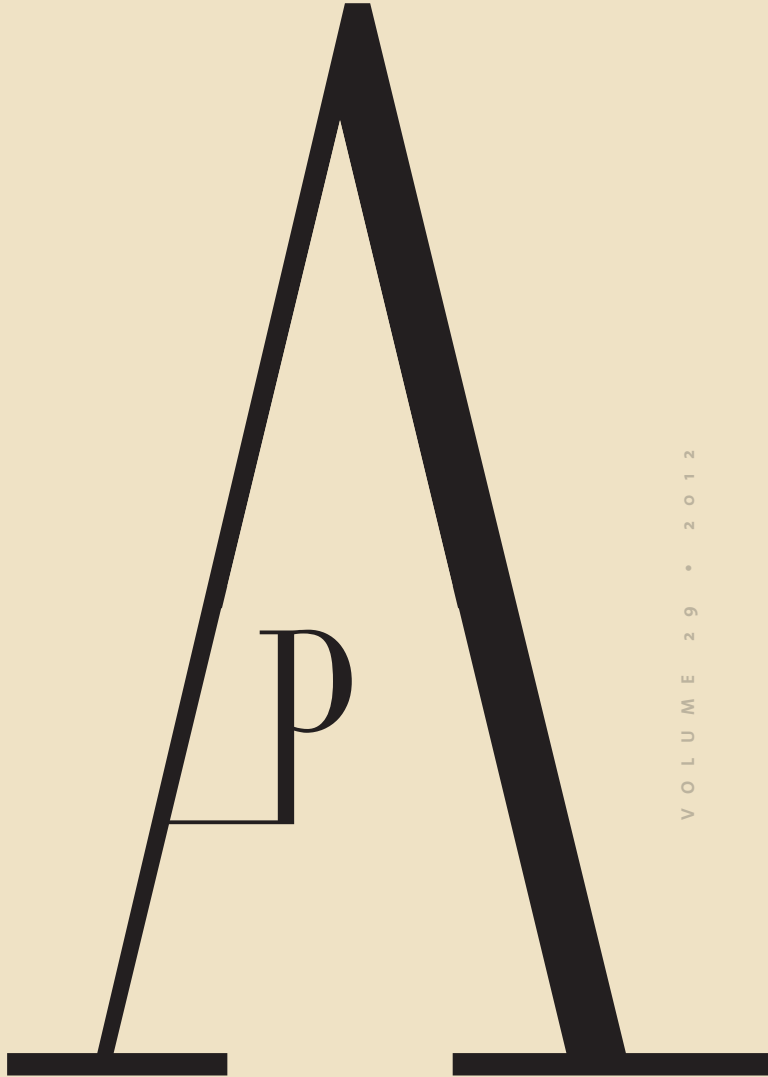


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The evolution of lactase persistence



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Abstract Lactase persistence has long been recognized as a striking example of human dietary adaptation to changes in food production habits. The observation that the prevalence of lactase persistence is positively correlated with the cultural history of dairying in human populations has led to the formulation of an evolutionary interpretation that became known as the culture-historical hypothesis. This hypothesis emphasizes the influence that culturally derived selection can have on human genes by assuming that the nutritional benefits of milk drinking during adult life are selectively advantageous in groups that rely on dairying to subsist. The recent demonstration of the molecular basis of lactase persistence provided a unique opportunity to test the basic predictions of the

Resumo A persistência da lactase é há muito considerada um exemplo notável de adaptação a modificações nos hábitos de produção de alimentos. A observação de que a frequência da persistência da lactase está positivamente correlacionada com a história cultural de produção de laticínios levou à formulação de uma interpretação evolutiva que ficou conhecida como hipótese “histórico-cultural”. Esta hipótese salienta a influência que a selecção mediada pela cultura pode ter no genoma humano, sugerindo que os benefícios nutricionais do consumo de leite na vida adulta são favorecidos por selecção natural em grupos que dependem deste alimento para subsistir. A recente demonstração da base molecular da persistência da lactase forneceu uma oportunidade única

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culture-historical hypothesis and to evaluate its merit relative to alternative explanations. Here, I present an overview of the evolutionary history of lactase persistence by focusing on the predictions of the culture-historical hypothesis, including the correlation between lactase persistence and pastoralism, the age of lactase persistence mutations and molecular evidences for natural selection. The places of origin and geographic diffusion of lactase persistence mutations are also discussed in the context of the population movements associated with the spread of pastoralism.

Key words Lactase persistence; natural selection; gene-culture co-evolution.

Introduction

Human culture has profoundly reshaped all major aspects of our environment, including diet, exposure to disease and capacity to occupy different ecoregions. Although some of these changes might have buffered selective pressures to which other organisms cannot escape, many cultural practices had important implications in the evolution of human genes that are known to be under positive selection (Durham, 1991; Laland *et al.*, 2010). Moreover, it is likely that the new selective pressures created by human culture, in combination with increasing numbers of available adaptive mutations

para testar as previsões da hipótese histórico-cultural e avaliar o seu mérito relativamente a explicações alternativas. Neste trabalho, apresenta-se uma revisão da história evolutiva da persistência da lactase focada nas previsões da hipótese histórico-cultural, incluindo a correlação entre persistência da lactase e pastoralismo, a idade das mutações responsáveis por este fenótipo e as evidências moleculares de selecção natural. Os possíveis locais de origem e a dispersão geográfica dessas mutações são também discutidos no âmbito dos movimentos migratórios associados à disseminação do pastoralismo.

Palavras-chave Persistência da lactase; selecção natural; co-evolução genes-cultura.

due to recent population growth, might have even accelerated human evolution during the last 40,000 years (Hawks *et al.*, 2007; Cochran and Harpending, 2009).

The current distribution of lactase persistence in human populations is an excellent example of the recent evolutionary impact of cultural change. Lactase is the enzyme that allows mammals to digest lactose, the main sugar in milk. In all mammals, the expression of the enzyme in the small intestine is down-regulated after weaning and decreases the ability to consume large amounts of milk after childhood (lactase restriction). Some humans, however, carry mutations that enhance lactase expression

throughout adulthood and are able to maintain during all their lives milk consumption habits that are typical of young mammals (lactase persistence). This variation in the ability to digest lactose is likely to have been strongly influenced by modifications in subsistence patterns favouring high milk consumption.

Here, I review the evolutionary history of lactase persistence by focusing on the processes that underlie its current genetic diversity in human populations.

The pattern

Lactase status, as assessed by a number of physiological tests, is highly variable in human populations (Ingram *et al.*, 2009; Itan *et al.*, 2010). Lactase restriction, the ancestral trait shared with other mammals, is the most common phenotype worldwide, including native populations from East Asia, Africa and America. Lactase persistence, the derived trait, is common in Europe and in some groups from Africa and the Middle East. The major aspect of this distribution is that lactase persistence is common mostly in populations with a longstanding tradition of milking pastoralism (Simoons, 1970; McCracken, 1971). In Europe, lactase persistence is most common (>80%) among the dairying-practising peoples from Scandinavia, Ireland and Great Brit-

ain, and gradually decreases towards eastern and southern areas of the continent, where frequencies can be as low as 15%, like in southern Italy and Greece (Figure 1). In Portugal, the frequency of lactase persistence, based on allele frequencies estimates, is about 60% (Coelho *et al.*, 2005), in pace with the position of the country in the European latitudinal cline (Figure 1). In Africa and the Middle East, lactase persistence is more irregularly distributed and may be very common (>60%) in pastoral groups like the Bedouin from Saudi Arabia, the Fulani from Cameroon, the Maasai from Kenya, and the Beja from Sudan, whose lactase statuses sharply contrast with those from their non-pastoralist neighbors (Ingram *et al.*, 2009; Itan *et al.*, 2010).

However, the correlation between lactase status and dairying traditions is globally imperfect, since several groups-like the Nuer from Sudan or the Herero from southern Africa have low frequencies of lactase persistence despite their reliance on milking pastoralism (Ingram *et al.*, 2009; Itan *et al.*, 2010). In these cases, there is a cultural adjustment to the inability to digest lactose, and milk is mainly consumed in processed forms with lowered lactose content, like cheese and yoghurt (Durham, 1991). For this reason, the prevalence of lactase persistence tends to be more strongly correlated with fresh milk consumption than with the degree of dependence on livestock

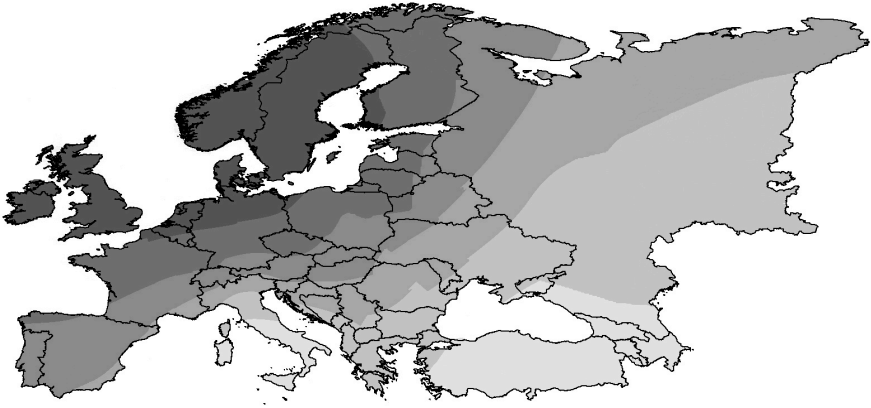


Figure 1. Interpolation map displaying the variation of lactase persistence frequencies in Europe, based on phenotype frequencies obtained with physiological tests, available at <http://www.ucl.ac.uk/mace-lab/GLAD/>. The darker the tone the highest the frequency. The maps were generated with the ArcGIS 9.2 program (www.esri.com).

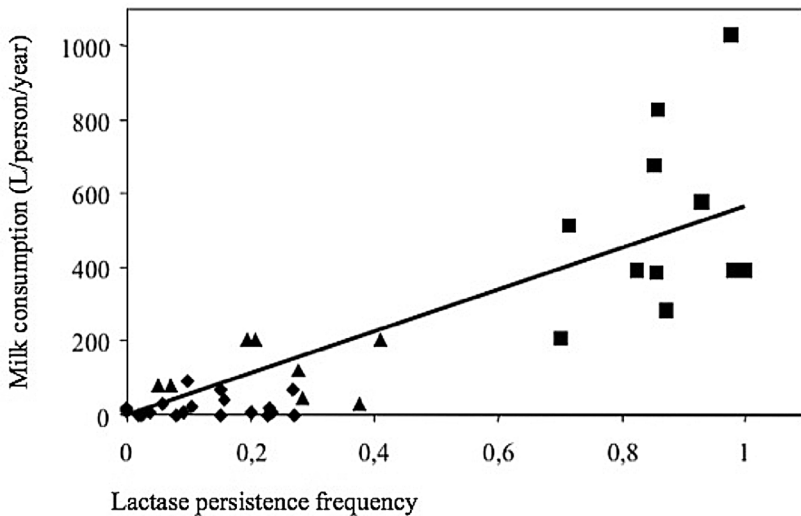


Figure 2. Correlation between lactase persistence frequency and milk consumption. Squares and triangles represent pastoral populations with high (>60%) and low (<40%) lactase persistence frequencies, respectively. Non-pastoral populations are represented by diamonds (based on data compiled by Durham, 1991).

(Figure 2). In any case, pastoralism seems to remain a necessary condition for lactase persistence to reach high frequencies in human populations (Holden and Mace, 1997).

The process

To explain the worldwide variation in lactase status, Simoons (1970) and McCracken (1971) proposed that lactase persistence was favored by natural selection in populations that depended on milk to fulfil their basic nutritional needs. According to this interpretation, also known as the culture-historical hypothesis, under conditions of milk dependence, lactase persistent individuals were able to enjoy an added nutritional benefit from high milk consumption that was not available to subjects with lactase restriction. In this scenario, the evolution of lactase persistence may be considered an example of human niche construction, since the selective environment leading to its increasing frequency was created by a cultural practice (Laland *et al.*, 2010; Gerbault *et al.*, 2011). Without the culture-mediated shift towards milk-based pastoralism there would be no fitness differences among individuals with contrasting lactase statuses (Durham, 1991).

The explanatory power of the culture-historical hypothesis depends on a

specific set of conditions. The first condition is, of course, the complete genetic determination of lactase status. If, for example, lactase activity could be physiologically induced by milk consumption, the Darwinian framework implied by the hypothesis would not be necessary to explain the observed distribution of lactase persistence (Flatz, 1987; Durham, 1991). Another important condition, as noted above, is milk dependence - the necessity to rely on milk to effectively obtain nutrients that are not available otherwise (Flatz, 1987). In this context, Johnson *et al.* (1974) have emphasized the dependency on *fresh* milk, since this is the sole dairy product that needs lactase to be digested. Fresh milk consumption would in turn require that milk was not processed into low-lactose products, or that fresh milk had specific nutritional benefits that are not found in processed products (Flatz, 1987; Durham, 1991). This condition may, however, be too strict, since it implies, with no justification, that milk processing habits inhibit people with lactase persistence from shifting towards increased fresh milk consumption and better explore this readily available food resource.

A major alternative to the culture-historical hypothesis (known as the reverse-cause hypothesis) suggests that the high frequencies of lactase persistence are unrelated with milk use and that dairying was adopted precisely by those popula-

tions that could digest lactose (McCracken, 1971). In this scenario, the differences in lactase persistence among human populations arose by genetic drift before major changes in subsistence patterns associated with the Neolithic (Nei and Saitou, 1986). Thus, the culture-historical hypothesis and the reverse argument have very different expectations concerning the temporal order by which cultural and genetic changes arose. Below, I will focus on the requirements of the culture-historical hypothesis and will highlight how its expectations better fit the observed data than explanations that are exclusively based on genetic drift and population history.

Inquiring the conditions: genetic basis for lactase persistence

The formal genetics of lactase status has been established by Sahi *et al.* (1973), who showed that lactase persistence is inherited as an autosomal dominant trait long before its molecular basis was fully understood.

More recently, Enattah *et al.* (2002) have shown that the T allele of a single nucleotide polymorphism (-13910 C/T) located 13.9 kb upstream of the lactase gene (LCT) was completely associated with lactase persistence in Finnish individuals. Subsequent studies have con-

firmed that the -13910*T allele could also explain the variation in lactase persistence in virtually all European populations (Ingram *et al.*, 2009; Itan *et al.*, 2010). However, with the exception of the Fulani and the Hausa from Cameroon, the -13910*T allele was found to be rare or absent in many Afro-Arabian communities where high frequencies of lactase persistence had been found in previous surveys based on physiological tests (Mulcare *et al.*, 2004; Ingram *et al.*, 2007; Enattah *et al.*, 2008). Later studies have identified additional variants located closely to the -13910 C/T polymorphism, which may explain variation in lactase status in those populations (Tishkoff *et al.*, 2007; Ingram *et al.*, 2007; Enattah *et al.*, 2008). Moreover, all these mutations lie on a region that is important for regulating lactase expression *in vitro* (Lewinsky *et al.*, 2005).

One of the new variants (-13907*G) is found among some Cushitic-speaking groups from Sudan and Ethiopia, but its association with lactase persistence is not robust (Tishkoff *et al.*, 2007; Ingram *et al.*, 2007; Figure 3A). Another variant (-13915*G) is clearly associated with lactase persistence in Saudi Arabians and Bedouins from Sinai with a tradition of camel milk consumption (Tishkoff *et al.*, 2007; Ingram *et al.*, 2007; Enattah *et al.*, 2008; Figure 3B). A third variant (-14010*C) is associated with lactase persistence in Nilo-Saharan and Afro-Asiatic-speaking

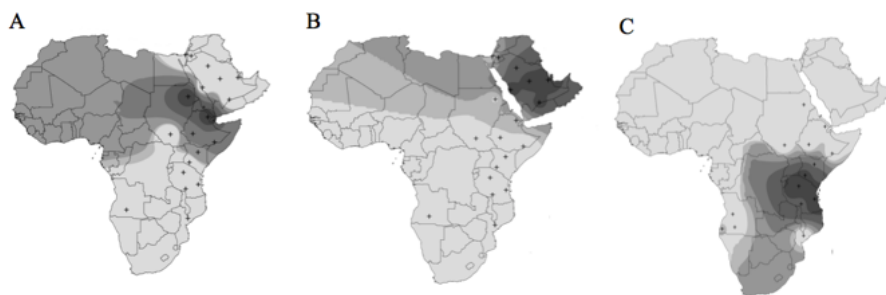


Figure 3. Interpolation map displaying frequency variation of the -13907*G (A), the -13915*G (B) and the -14010*C (C) alleles, using data available at <http://www.ucl.ac.uk/mace-lab/GLAD/>. The darker the tone the higher the allele frequency. Sampled locations are marked with a cross. In the unsampled areas, allele frequencies were deduced assuming a linear decrease in frequency with distance from the area with highest frequency. The maps were generated with the ArcGIS 9.2 program (www.esri.com).

pastoral populations from Kenya and Tanzania (Tishkoff *et al.*, 2007; Figure 3C).

Milk dependence

The nutritional importance of milk is closely linked to the major dietary shifts associated with the adoption of agriculture. It is increasingly recognized that early agriculturalists had much less balanced diets than hunter-gatherers, being chronically exposed to vitamin and protein shortages (Diamond, 1987; Cochran and Harpending, 2009). In this framework, milk must have become an invaluable food resource that could be obtained without the sacrifice of domesticated animals. Moreover, cattle

raising is probably one of the most effective ways to subsist in marginal environments, even for groups where lactase restriction predominates, as shown by the nearly exclusive pastoral specialization of many semi-nomadic peoples that dwell in the arid regions of Africa and Arabia (Flatz, 1987; Holden and Mace, 1997). Cook and Al-Torki (1975) have additionally proposed that, in those regions, fresh milk could be an important source of uncontaminated fluids that could not be obtained in processed dairying products. Unsurprisingly, it is the Afroarabian pastoral groups that are consensually thought to meet the conditions required for natural selection to act in the framework of the culture-historical hypothesis (Flatz, 1987; Durham, 1991; Gerbault *et al.*, 2009). In contrast,

the idea that general nutritional milk dependence can explain the patterns of lactase persistence in northern Europe, where mixed farming systems predominate, is more controversial (Flatz, 1987; Durham, 1991). To account for the specific advantages of drinking fresh milk in high-latitude regions in Europe, Flatz and Rotthauwe (1973) have proposed that individuals with persistent lactase activity could benefit from the ability of lactose to stimulate calcium absorption. According to this “calcium absorption” hypothesis, lactase persistence could mitigate the risk of rickets and osteomalacia in populations that are prone to vitamin D deficiency due to low solar irradiation. This model can additionally explain the significant correlation between lactase persistence frequencies and latitude in Europe (Flatz, 1987; Durham, 1991; Figure 1). To better assess the specific selective advantages of lactase persistence, Holden and Mace (1997) analyzed the influence of milk-based pastoralism, aridity and solar radiation, by using phylogenetic comparative methods designed to overcome the confounding effects of shared ancestry among the groups being compared. They concluded that lactase persistence did co-evolve with pastoralism, but found no evidence for additional selection pressures due to aridity or high latitude. This conclusion is closer to the original formulations of the culture-historical model pointing to the

general nutritional benefit of milk-drinking as the single selective mechanism in dairying peoples from widely different economic and climatic conditions.

More recently, Gerbault *et al.* (2009) used geographically explicit simulations to study the spread of lactase persistence over Europe and found that, in accordance with the calcium absorption model, selection pressures had to increase with latitude to explain the observed patterns of variation (see discussion below). These contrasting results show that hard evidence for the specific selective advantage of lactase persistence is more difficult to find than initially though.

Assessing the expectations: chronology of the evolution of lactase persistence

The culture historical model and the reverse argument have very different expectations about the chronology of major events influencing the evolution of lactase persistence. In the selection framework of the culture-historical hypothesis, dairying practices preceded high lactase persistence frequencies, and the age of lactase-persistence mutations can be as recent, or even more recent than milking-pastoralism. Differently, according to the reverse hy-

pothesis, lactase persistence should be much older to allow for its frequency to increase by genetic drift (Nei and Saitou, 1986). For example, using the formulations derived by Kimura and Ohta (1973) it would take on average 137,500 years (5,500 generations) for a mutation to reach a 0.5 frequency (much lower than in many pastoral communities) under neutrality, assuming an effective population size N_e of 2000 (much lower than usually assumed). Increasing the allele frequency and/or the population size to more realistic values would considerably extend this time.

By using a maximum-likelihood approach to analyze the worldwide distribution of lactase persistence, Holden and Mace (1997) found that the favored direction of evolutionary change included an ancestral “no-milking/no-lactase persistence” stage, followed by an intermediary phase with milking and no-lactase persistence, and a final association between milking and lactase persistence, as predicted by the culture historical model.

Moreover, we have estimated the absolute age of the -13910*T allele to be around 8,000 years (Coelho *et al.*, 2005), which is consistent with the time frame for the beginning of dairying practices, and too recent to be explained by the reverse argument. Briefly, our approach consisted in the use of microsatellite markers closely linked to the -13910 C/T

polymorphism as molecular clocks to date the -13910 C*T mutation. The dating principle we used explores the fact that the original lactase persistence mutation became associated with a single allele at each linked microsatellite locus (highest intra-allelic homogeneity). With time, mutations at the microsatellite loci and recombination between the microsatellites and the -13910 site are expected to decrease the initial intra-allelic homogeneity and this decay in intra-allelic homogeneity, can be used to estimate the age of the mutation (Figure 4).

Age estimates for alleles -14010*C (4,000-8,000 years; Tishkoff *et al.*, 2007) and -13915*G (~4,000 years, Enattah *et al.*, 2008) are also very recent and consistent with the predictions of the culture-historical hypothesis. Moreover, Burger *et al.* (2007) provided additional evidence for a recent origin by showing that the -13910*T allele was absent in 8 ancient DNA samples from Neolithic archeological sites located in regions where lactase persistence is common nowadays. Further evidence is provided by recent studies in 14 Neolithic samples from Scandinavia (Malmström *et al.*, 2010) and 26 Neolithic samples from the Basque Country (Plantinga *et al.*, 2012), that have found that frequencies of 13910*T 5,000 to 4,200 years ago were much lower than currently observed at the same sites.

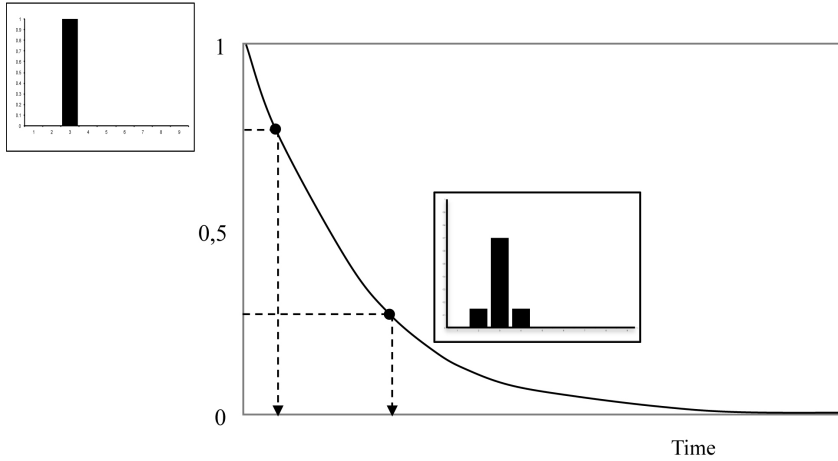


Figure 4. Schematic of the principle used to date a mutation through linked microsatellite variation. The graphic shows the overtime decay of intra-allelic homogeneity due to microsatellite mutation according to a stepwise mutation model (varying from 1 to 0 in the ordinate). The insets show the allele frequency distributions in a linked microsatellite locus *within* chromosomes bearing the dated mutation, at different time periods. In this case, only one microsatellite is used and the intra-allelic homogeneity is simply measured by the frequency of the microsatellite allele that became originally associated with the mutation, within chromosomes bearing that mutation.

Molecular signatures of natural selection

Since the culture-historical model postulates a rapid increase in the frequency of lactase persistence-associated alleles, strong signatures of positive selection are expected to be found in loci associated with the trait.

In accordance with this expectation, we and others observed a significant departure from neutrality, by showing that the -13910*T allele was too young to reach its

present high frequencies without the aid of selection (Bersaglieri *et al.*, 2004; Coelho *et al.*, 2005; Figure 5). Similar signatures of selection were found around the -14010 and the -13915 sites, providing a striking example of convergent evolution. Consistent with these findings, the regions around the LCT gene were found to carry one of the strongest signals of selection of the human genome in genome-wide scans for natural selection (Voight *et al.*, 2006).

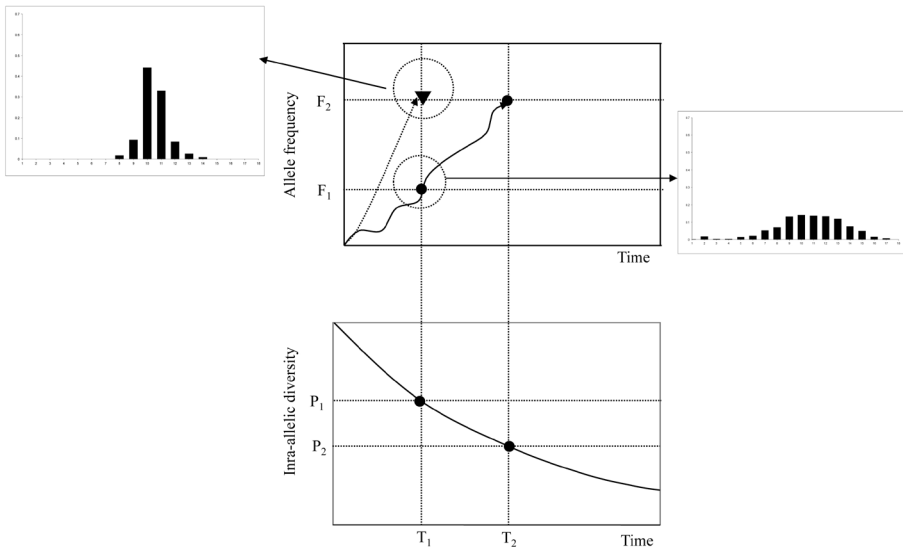


Figure 5. Schematic of the principle used in neutrality tests for lactase persistence. The graphic on the top shows the overtime increase in frequency of a neutral (filled trajectory) and a selected allele (dotted trajectory). The graphic on the bottom shows the overtime decay of intra-allelic homogeneity. A neutral allele is expected to reach frequency F_1 at time T_1 (F_1 , T_1) and frequency F_2 at time T_2 (F_2 , T_2). Natural selection breaks the expected relationships between age and frequency by making young favored alleles reach frequencies that are higher than expected under neutrality (F_2 , T_1). The insets show the allele frequency distributions in a linked microsatellite locus *within* chromosomes bearing favored and neutral alleles. Note the lower diversity (younger age) associated with the favored allele and the highest diversity (older age) associated with the ancestral, non-selected allele.

Milk ways: the dispersal of lactase persistence

Although selection and population history are sometimes viewed as opposing evolutionary factors, it is clear that

demographic processes have played a major role in the current distribution of selected alleles (Coop *et al.*, 2009). Loci under selection may thus be important tools to reconstruct recent migration events.

The geographic segregation of the three major alleles associated with lactase persistence (-13910*T, -14010*C and -13915*G) provides a unique opportunity to trace the migratory movements of peoples with different pastoralist traditions. The -13910*T variant is the most widespread lactase-persistence-allele. So far, it has been found in Europe, Middle East, North Africa, the Fulani from Cameroon, northern India and Pakistan, as well as the Urals and the Caucasus Mountains. The distribution and age of this variant is most consistent with a Eurasian origin, followed by regional introductions in more peripheral areas like Africa. When linked to well documented migrations, some of these movements may be relatively easy to infer. For example, Myles *et al.* (2005) proposed that the introduction of the -13910*T variant in North Africa could be explained by the Neolithic spread of ovicaprid Berber-speaking herders from the Middle East. Moreover, the presence of the -13910*T allele among the Cameroonian Fulani may reflect past admixture with Berber nomads in the original Fulani area around Senegal, before the Fulani diaspora towards East across the Sahel (Mulcare *et al.*, 2004; Curtin *et al.*, 1995). However, the precise geographic origin of the -13910*T allele in Eurasia is more difficult to infer. Based on the geographic distribution of haplotype variation around the -13910*T lineage, Enattah *et al.* (2007) proposed an

origin centered in the Urals. More recently, Itan *et al.* (2009) simulated the spread of lactase persistence in Europe and western Asia and inferred that it is more likely that the -13910*T allele first underwent selection between central Europe and northern Balkans about 7500 years ago. Furthermore, these authors concluded that interaction between demographic processes and constant selection pressures was enough to explain the patterns of lactase persistence in Europe (Figure 1), and that the trait was not more favored in northern latitudes. This conclusion is quite different from that of Gerbault *et al.* (2009), who found evidence for additional selective pressures in high latitude, in accordance with the calcium absorption hypothesis (see above).

The age and geographical distribution of the -13915*G allele (Figure 3B) suggest that this variant originated in the Arabian Peninsula around 4,000 years ago and then spread northwards into the Middle East and westwards into Africa, probably in association with the domestication of the Arabian camel (Enattah *et al.*, 2008).

The -14010*C allele is particularly frequent in non-Bantu peoples from Kenya and Tanzania (Figure 3C). The oldest time age estimates for this variant (6,000-7,000 years; Tishkoff *et al.*, 2007) were found in these populations, suggesting that it is associated with the emergence of the pastoral tradition of the Great Lakes which relies heavily on cattle raising. In-

terestingly, we found the -14010*C allele at relatively low frequency (6%) in the Kuvale, a Herero-speaking group from southern Angola (Coelho *et al.*, 2009). Our observation may provide evidence for a direct link between the relatively isolated southwestern Africa pastoral scene and the major cattle herding centers of East Africa, as proposed by early Anthropology scholars studying southwestern Africa (Estermann, 1961). However, we favor a more indirect trajectory whereby Pre-Bantu Khoe-Kwadi (Central Khoisan) speaking pastoralists originating in East Africa carried the -14010C variant into southwestern Africa and subsequently transferred it by admixture to adjacent Kuvale pastoralists (Coelho *et al.*, 2009; Rocha, 2010). Such a Pre-Bantu pastoralist migration is also supported by a recent evaluation of Khoe-Kwadi in the context of click languages from southern Africa (Güldemann, 2008).

Conclusions

According to the available evidence, in the last 2,000-12,000 years at least three variants (-13910*T, -14010*C and -13915*G) associated with lactase persistence have evolved independently, reaching high frequencies in diverse human groups with long tradition of dairying pastoralism. This pattern, together with the molecular signatures associated with each

variant, provides one of the clearest examples of recent natural selection in the human genome, broadly validating the culture-historical hypothesis formulated more than 40 years ago.

More specific aspects of the hypothesis, like the precise nature of the selective advantage conferred by lactase persistence and the effects of demography and selection in spreading this trait, remain to be fully elucidated. Studies using geographic explicit simulations to address the mode and direction of advance of lactase persistence mutations provide excellent examples of the usefulness of new computational methods to address these questions and to reconstruct the evolutionary history of selected traits (Gerbault *et al.*, 2009; Itan *et al.*, 2009).

Recent additional studies have pointed out that the three major lactase persistence variants identified so far do not explain all the observed phenotype variation (Itan *et al.*, 2010). Thus, it is likely that additional surveys based on careful genotype/phenotype contrast will uncover additional variants in the future. The abundance of different candidate mutations for lactase persistence highlights the need for fine-scale geographic sampling when searching for new genetic variants under selection (Tishkoff *et al.*, 2007). In particular, more care should be taken to mitigate the current shortage of African samples in public repositories of human genetic variation.

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