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The Palaeopopulationgenetics of Humans, Cattle and Dairying in Neolithic Europe

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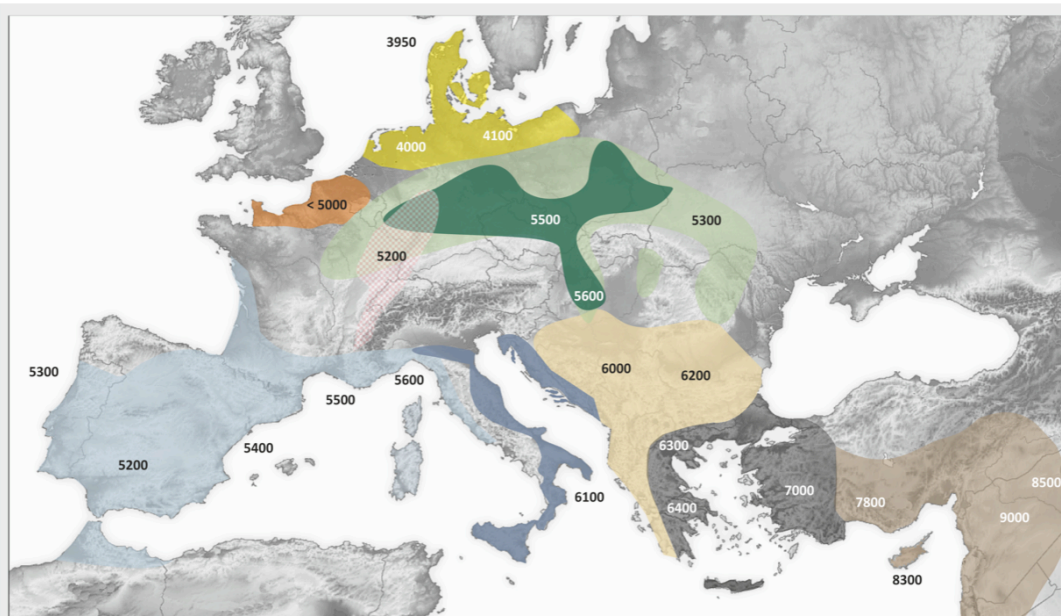[1] *Institut für Anthropologie, AG Palaeogenetik, Johannes Gutenberg-Universität, Germany*
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15.1 INTRODUCTION

The term 'Neolithic' most often refers to a package of features including permanent settlements, agriculture, pottery and animal husbandry. This definition is valid for an area between Western Anatolia and Central Europe, but approaches the limits of its utility in many other parts of the world. For example, pottery has been found in pre-Neolithic Africa, and signs of the 'Neolithic package' are found in Mesolithic cultures of the Northern Europe and the Baltic region. The term 'Neolithic' is also frequently used purely chronologically and so has been applied to parallel hunter-gatherer societies who are culturally 'Mesolithic', even if chronologically they live in a Neolithic phase. Such variability in definitions and nomenclature means that the term 'Neolithic' does not describe a universal and uniform process, a point that should be taken into consideration not only in archaeology, but also in studies of population biology.

Humans first started managing goats, sheep, pigs and cattle some 11,000 to 10,000 years ago in a region between the Levant, the Zagros mountains and Central Anatolia (Zeder, 2008). After 7,000 calBC, archaeological signatures of a Neolithic package first appeared outside of the core region (Figure 15.1), in archaeological sites in southern and western Anatolia, and seem to have spread there from central Anatolia (Özdogan and Basgelen, 1999). By around 6,400 calBC, a Neolithic package appears in the Greek Aegean (monochrome phase in Thessaly and the Peleponnes and proto-Sesklo in other regions), and simoultaneously around the Marmara Sea, at the north-west tip of Anatolia and in Eastern Thracia.

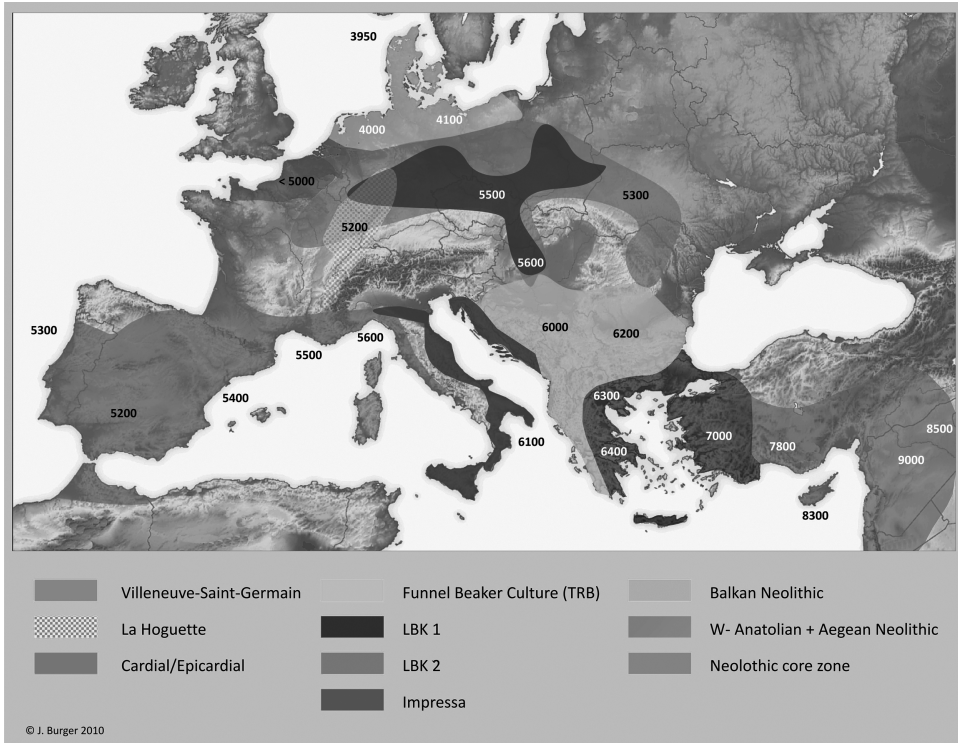|		
Villeneuve-Saint-Germain	Funnel Beaker Culture (TRB)	Balkan Neolithic
La Hoguette	LBK 1	W- Anatolian + Aegean Neolithic
Cardial/Epicardial	LBK 2	Neolithic core zone
	Impressa	

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Figure 15.1   Chronological spread of the Neolithic. Numbers give the approximate earliest dates of the Neolithic in years before Christ (calBC). (See Plate 15.1 for a colour version of this image)

At this point, pottery style analysis indicates two culturally distinct but geographically overlapping trajectories for the spread of Neolithic culture in Europe: a so-called 'Mediterranean Route' and a 'Danubian/Balkan Route'. The former spread via southern Italy and the Adriatic (so-called Adriatic Impressa) to the islands of the Tuscan archipelago and later on to the south of France and the Iberian Peninsula (the so-called Cardial Ware culture). In the following, we focus on the so-called 'Danubian/Balkan Route', which is better studied from a palaeogenetic point of view.

After an initial phase of monochrome ceramics, a multitude of archaeologically defined cultures develop on the Balkan Peninsula and in the Carpathian Basin. These cultures can be broadly subdivided into the Karanovo I complex and the Starčevo-Körös-Çris complex. The Linearbandkeramik culture (LBK) spreads rapidly from what is believed to be its Starčevo forerunners in Central Europe from about 5,500 calBC (Pavuk, 2005). Prior to 4,100 calBC, Neolithic culture remains confined to these limits and only later expands to the North German lowlands and other regions of Northern Europe (Figure 15.1).

In this chapter, we compile the findings from the major palaeogenetic studies of the European Neolithic, published in recent years. We include studies of humans as well as of domestic animals, which can act as proxies for human population dynamics. First, we outline the differences between modern model based approaches and classical phylogeograpic interpretation of palaeogenetic data in population history inference. We then summarize

and discuss how palaeogenetic studies have been used to examine key questions on the demographic and evolutionary histories of Neolithic cattle and humans, including recent studies on the spread of lactase persistence.

15.2 THE AIM OF PALAEOGENETIC STUDIES IN THE CONTEXT OF THE NEOLITHIC TRANSITION IN EUROPE

Following the pioneering work of Luca Cavalli-Sforza in the 1970s and 1980s (Cavalli-Sforza *et al.*, 1986; Bowcock *et al.*, 1987), a series of studies have been performed over the last 20 years that attempt to infer human demographic history through patterns of genetic variability in modern populations. In the early days of research, classical genetic markers and later mitochondrial DNA (mtDNA) were the main contributors to this field, particularly through the interpretation of summary patterns of variation, such as Principle Components Analysis (Cavalli-Sforza, Menozzi and Piazza, 1996) and phylogeographic (Avise, 2000) analysis of hypervariable region I (HVR I) sequences (Richards *et al.*, 2000).

A central tenant of phylogeographic inference is that the nodes of major clades on an inferred phylogenetic tree, or a network, correlate with demographic events in time and space. However, this approach has been criticized (Goldstein and Chikhi, 2002; Nielsen and Beaumont, 2009, Beaumont *et al.*, 2010, but also see Templeton, 2009, 2010) and studies based on computer simulations (Knowles and Maddison, 2002; Panchal and Beaumont, 2007) have highlighted major problems with the more systematic incarnations of phylogeographic inference (Templeton, 1998). While phylogeographic inference has dominated palaeogenetics over the last 15 years, explicit model-based statistical inference approaches – particularly those employing coalescent theory (Griffiths and Tavare, 1994) – have developed apace in the field of population genetics. Coalescent theory is a retrospective model of gene genealogies for a sample and permits the probabilities of lineages joining (when viewed backwards through time) to be calculated under a range of different demographic conditions (e.g. population constancy, growth, etc.). These approaches emphasize the stochastic nature of the genealogical process in populations and recognize that very different 'gene trees' can arise from very similar demographic histories, and vice-versa. In other words, the gene tree and the demographic history of a population are, to a greater or lesser extent, decoupled and, in a sense, the gene tree can be thought of as a nuisance parameter. Lineage sorting – the phenomenon whereby lineages first coalesce not with lineages in the same population, but rather with lineages in a related population, illustrates the dangers of misinterpretation if the demographic history of populations is inferred from a phylogenetic tree/network. Similar problems arise when interpreting summary patterns of genetic variation, such as Principal Components Analysis (Cavalli-Sforza, Menozzi and Piazza, 1996). For example, different gradients of Principle Components in space have been interpreted as signatures of past migration events. Recent simulation studies have shown that components may form predictable patterns in space, but that these do not necessarily represent demographic episodes, if indeed they have any sensible interpretation at all (Novembre and Stephens, 2008).

The challenge in population genetic inference is to understand, in a statistical framework, what historical scenarios could have given rise to the data, and in relation to the geographical location of samples. The solution to this problem is to explore (usually by simulation) different historical scenarios and search for the conditions under which the data, or some description of the data, has the highest probability of arising. This approach not only allows

parameters of an evolutionary model to be estimated but also allows formal statistical comparison of different models of demographic history. Unfortunately this is not a trivial task, because appropriate models need to be used that are sufficiently complex to reflect the important processes that shape patterns of genetic variation, but are not so complex that they are difficult to implement or when implemented, can explain any data. However, important developments such as Approximate Bayesian Computation (Beaumont, Zhang and Balding, 2002) make this task much easier as it allows estimation of parameters of a model, and comparison of different models, without the need to compute exact probabilities of data under specified models.

Because of problems of inference when using DNA data from modern individuals, as outlined above, the most direct way to examine demographic and evolutionary history is to obtain ancient DNA data directly from archaeological skeletal populations in the timeframe under study. This does not mean that the outlined inferential challenges disappear altogether. But for many of the questions archaeologists are interested in, such as population continuity vs. admixture, the task becomes somewhat easier. Accordingly, by applying palaeogenetic methods, archaeological hypotheses concerning the spread of people, raw materials, artefacts and pottery styles (and thereby of 'cultures') can be tested. Although palaeodemography is often taken as representing the biological analogue of culture, the extent of any correlation between genetic and material culture data may actually be widely different for different episodes in prehistory.

15.3   THE IMPORT AND MOVEMENT OF DOMESTIC ANIMALS

The early Neolithic of the Near East and Anatolia is characterized by the domestication of animals including goats, sheep, pigs and cattle, at the latest by 8,500 calBC (Zeder, 2008). The transformation of wild forms of these four species into domestic animals is, alongside sedentism and crop (cereals and legumes) agriculture, a characteristic of early Neolithic cultures. This transformation was most likely a long process and appears to have taken place in the core regions of the Near East and Anatolia, long before the spread of agriculture into western Anatolia and southeast Europe. As both the morphological and the population-genetic consequences of the management of wild animals are unlikely to differ significantly from those of full domestication, uncertainty persists on where and when domestication in the full zoological sense actually took place. Foreseeably, palaeogenetic analyses of gene variants associated with the 'domesticated' phenotype will provide crucial information on this question in the future. The information we have so far is mainly from non-coding mitochondrial DNA, which does not tell us much about the phenotype of an animal but should contain information on its demographic history. The best investigated species in Neolithic times is cattle. Initial studies on the mitochondrial variability of present-day cattle in the Near East, Anatolia, North Africa and Europe showed that the variability in the vicinity of the postulated Neolithic domestication process is higher than that in Europe and/or North Africa (Troy *et al.*, 2001). The prevalence of T and T3 lineages in Europe and of T1 lineages in North Africa is consistent with the idea of an export of early domestic animals from the Neolithic core zone to Europe and North Africa. Nevertheless, this dataset can be interpreted in a variety of ways (e.g. the reduced variability in Europe could be the result of historical breeding practices). In order to further examine this question, skeletal remains from the Neolithic period in central, north and south-east Europe were examined.

It was found that the genetic variability of today's cattle is similar to that of early Neolithic cattle (Bollongino *et al.*, 2006). But a deeper understanding of these results requires assessment of whether European domesticated cattle were introduced into Europe from the Near East or were descended wholly or in part from wild European cattle.

Morphologically, European wild cattle (*Bos primigenius*), otherwise known as *aurochs*, differ from domestic cattle (*Bos taurus*), mainly in terms of the size of skeletal elements. This represents a methodical problem as individual male taurine cattle and individual female aurochs have a significant overlap in size of all skeletal elements. Therefore, in order to be able to study known European aurochs, Pleistocene or Mesolithic Holocene specimens must be analysed. So far, all known aurochs identified carry mtDNA haplotypes that are classified phylogenetically as P or E. These lineages are distinct from those found in Anatolian and Middle Eastern taurine cattle (which are mostly T- and Q-types) (Scheu and Bollongino, unpublished data). As all known wild cattle in central and northern Europe carry P-types, or to a lesser extent E-types (Edwards *et al.*, 2007), the T- and Q-types have been proposed to originate from outside Europe, probably from early Neolithic domesticates in Anatolia or the Near East. Using coalescent simulations conditioned on both ancient and modern DNA sequences, it should be possible to estimate key parameters of the domestication history of these earliest cattle, such as the effective size of the population at the time of domestication.

Admixture between imported domestic cattle and indigenous European aurochs can, to a first-order approximation, be excluded in the case of central Europe (Bollongino *et al.*, 2006; Scheu *et al.*, 2008; Edwards *et al.*, 2007), but this remains a plausible scenario for other parts of Europe (Beja-Pereira *et al.*, 2006). In south-eastern Europe, most aurochs bones from Early Neolithic sites are of relatively small-sized specimens and hence the differentiation between *Bos taurus* and *Bos primigenius* on the basis of archaeozoological criteria is problematic.

With respect to pigs, extensive ancient and modern mtDNA data have been analysed and interpreted in a phylogeographic framework to indicate multiple independent centres of domestication. (Larson *et al.*, 2005). We consider the latter scenario unlikely, and given that wild boar is very common in Europe, it is more likely that progressive admixture between local wild boar and imported (domesticated) Anatolian pigs is responsible for the pattern observed (Larson *et al.*, 2007). However, as with the other domestic species – sheep and goat – more extensive coalescent modelling studies need to be performed to fully and explicitly examine models of the spread of these animals in Europe. Thus, there is evidence that both pig and cattle have been imported to south-east Europe and then spread over the continent within three millennia, with pigs being mixed with local wild boar and cattle remaining maternally almost unmixed with local aurochs. With regards to sheep and goat, there is little doubt that they were imported from the Near East and/or Anatolia into Europe, as there are no wild progenitors of these species in Europe.

The fact that four different species were disseminated over such a long time and moved into such a large area previously free of agriculture, and also considering the difficulties of breeding and transport in a new ecological niche, the question arises as to which cultural and technical abilities were necessary for the spread of stock keeping. Certainly, the rapid dissemination of all four species over the continent is unthinkable without favourable social and economic conditions and it is tempting to speculate that the Mesolithic-Neolithic transition in Europe involved some professional trade in domestic animals. Furthermore, it is clear that the four domestic species did not migrate without humans. But the extent to which humans moved into Europe continues to be debated (Barbujani, Bertorelle and Chikhi, 1998; Torroni *et al.*, 2000, Chikhi *et al.*, 2002, Dupanloup *et al.*, 2004; Belle, Landry

and Barbujani, 2006). As mass migration of Neolithic farmers seems at first glance unlikely, the predominant view of continental archaeologists over the last few decades was that a few pioneers were responsible for the dissemination of Neolithic agriculture (Lüning, 2007) and that Neolithic economy and lifestyle were adopted by local Mesolithic hunter-gatherer populations (Kind, 1998; Gronenborn, 1997). As we discuss in the following section, recent palaeogenetic evidence challenges this traditional view, at least for Central and Northern Europe.

15.4   THE DEMOGRAPHY OF LATE HUNTER-GATHERERS AND EARLY FARMERS

Little is known about the palaeogenetics of late Glacial and early Holocene human populations in Europe. The first molecular genetic study of early LBK skeletons from Central Europe – all dating to between 5,500 to 5,000 calBC – did little to reveal the origin of Europe's first agriculturists, but did produce a surprising result. Although the LBK was the pioneer Neolithic farming culture of central Europe, their mitochondrial lineages appear to have been severely diluted over the subsequent millennia and some of them are rare in modern Europeans (Haak *et al.*, 2005). Two main explanations have been offered to account for this observation. The first, which is consistent with some archaeological theories (Lüning, 2007), is that the early LBK farmers were small groups of pioneers who were later replaced by surrounding hunter-gatherers. The second scenario is that these pioneer LBK farmers were to a large extent replaced by subsequent waves of farmers carrying different mtDNA lineages. However, currently neither of these scenarios receives support either from archaeology or palaeogenetics. In a more recent study and using coalescent simulations, Bramanti and colleagues (2009) further confirmed the observation that the LBK farmers were not the sole direct ancestors of modern Central Europeans, but to date, the missing continuity between the early farmers of central Europe and modern Europeans remains to be explained. It is likely that a combination of a series of demographic processes, which post-date the initial colonization of central Europe by the pioneer LBK farmers, is responsible for this lack of genetic continuity. Future analysis of palaeogenetic data from later periods, including spatially explicit serial modelling of heterochronous aDNA data, will allow us to assess the effects of these demographic processes in more detail.

As part of the same study, Bramanti and colleagues (2009) also examined mitochondrial DNA from skeletons of European hunter-gatherers from Upper Palaeolithic, Mesolithic, and Neolithic periods. The individuals examined came from different locations in southwest Germany, northern Germany and the Baltic region. Specimens from various archaeological periods were considered as a single group of 'hunter-gatherers' because of their similar subsistence strategies, even though it is not certain if they belong to any single population in a narrower biological sense. As a group, they represent the descendents of the hunter-gatherers that re-colonized central and northern Europe from southern European refugia after the end of the last Glacial maximum, around 20,000 BP. The following major climatic/environmental changes during deglaciation, and corresponding re-colonization of the biota in northern Europe, meant that large parts of this region were also re-occupied by human populations. These hunter-gatherers are probably the descendents of the first anatomically modern humans to settle in Europe around 45,000 years ago. The authors have shown that, in genetic terms, the LBK sample and the hunter-gatherer sample were indeed significantly different, as expressed

by a comparatively high F_{ST} value (0.163). F_{ST} is a measure of genetic difference between populations and quantifies the proportion of genetic variation in a set of population samples that is attributable to differences between those samples. For comparison, the observed F_{ST} (0.163) between the two prehistoric groups was higher than the highest values found between similarly sized samples from a set of global populations ($F_{ST} = 0.133$). Serial coalescent simulations – that is coalescent simulations where a simulated lineage can be sampled at different points in time – were carried out in order to determine whether or not the difference between the LBK sample and the hunter-gatherer sample, as measured by F_{ST}, could be explained under the null-hypothesis of population continuity. Because genetic differences between populations in space and time are determined by a variety of factors, including ancestral population sizes, it was necessary to perform the simulations under a wide range of assumed combinations of population size at the start of the Neolithic and the Upper Palaeolithic colonization of Europe some 45,000 years ago. These simulations revealed no continuity scenarios in which a significant proportion of the simulated F_{ST} values were the same or higher than those actually observed. This allowed direct continuity between the hunter-gatherer sample and the LBK sample – the null-hypothesis – to be rejected. In another palaeogenetic study on the genetic contribution of late hunter-gatherers to modern Europeans, Malmström and colleagues (Malmström *et al.*, 2009) showed that the peoples of the last major hunter-gatherer complex in Europe, the Pitted Ware culture of southern Scandinavia, were unlikely to be the direct ancestors of modern Swedes, Norwegians or Saami. They did, however, show that direct continuity between the Pitted Ware peoples and those of the eastern Baltic region was possible. This does not mean that they actually were the ancestors of modern Eastern Baltic populations, as it can also – and more plausibly – be explained by common ancestry between the sampled populations. It is intriguing to note that the predominant lineage found in both the central European and the Scandinavian hunter-gatherers, haplogroup U (Bramanti *et al.*, 2009; Malmström *et al.*, 2009), is the same as that observed in a single alleged Upper Palaeolithic Russian burial from the site of Kostenkii 14 (Markina Gora), which is indirectly dated to approximately 30,000 years ago (Krause *et al.*, 2010), although in our opinion it is hard to exclude contamination in such a study (Pääbo *et al.*, 2004).

The study by Bramanti and colleagues (2009) was not the first genetic study to address the question of hunter-gatherer contribution to early European farming populations or to modern Europeans (Barbujani, Sokal and Oden, 1995; Barbujani, Bertorelle and Chikhi, 1998; Chikhi *et al.*, 1998; Richards *et al.*, 2000; Semino *et al.*, 2000; Chikhi *et al.*, 2002; Dupanloup *et al.*, 2004; Currat and Excoffier, 2005). However, these previous studies were all based on modern genetic data – where inference requires many explicit, and in some cases implicit assumptions. Also, most were aimed at obtaining estimates of admixture between hunter-gatherers and incoming farmers using questionable proxies for ancestral source populations (specific lineages in the case of those studies based on phylogeographic inference, modern population samples in the case of those studies that used explicit modelling). Therefore, given the uncertainties of the inference process (see above), the null-hypothesis of population continuity remained to be formally rejected. Because Bramanti and colleagues (2009) sampled directly from ancient populations living around the time of the transition from hunter-gathering to farming, they – for the first time – were able to formally reject continuity. It is likely that modern Europeans are a product of admixture between these two groups – Central European hunter-gatherers and LBK farmers – and probably other ancestral source populations. But those other ancestral source populations remain to be identified and represented with ancient DNA data. Without such data, any estimates of

admixture proportions in early farmers, late Neolithic populations and any subsequent European peoples up to the present day, will remain unreliable.

Since the first LBK farmers of central Europe were clearly not the direct descendents of local hunter-gatherers, they must have migrated in from another region. There are, as yet, no palaeogenetic data to indicate the most probable region of origin of the early LBK farmers. From an archaeological perspective, the most plausible region of origin is around the area of Lake Balaton in present-day Hungary (Pavuk, 2005; Figure 15.2), since this is the region (along with south-west Slovakia) where the LBK first developed around 5,700 calBC from the predecessor Starčevo culture. From 5,500 calBC until the end of its initial expansion phase around 5,300 calBC, the LBK culture spread rapidly across central Europe, with settlements quickly reaching from the Rhine valley to eastern Poland. By around 5,000 calBC, the LBK culture had expanded further, stretching from the Paris Basin through to the Ukraine. Since the LBK culture spread from a clearly restricted region, and because at least the early LBK farmers investigated were such highly mobile immigrants, sampling ancient skeletal material from the Lake Balaton core region will be essential to better understand the genetic origins of Central Europe's first farmers. Unfortunately, there is currently an almost complete absence of skeletons from the oldest phase of the LBK, that is between 5,600 and 5,400 calBC (Lüning, 2005).

If the scenario of a migration from the north-western part of the Balkans westwards to central Europe during the Neolithic could be examined using ancient DNA data, then the question that immediately arises is whether it was a single event (perhaps of a mass migration) or only one of
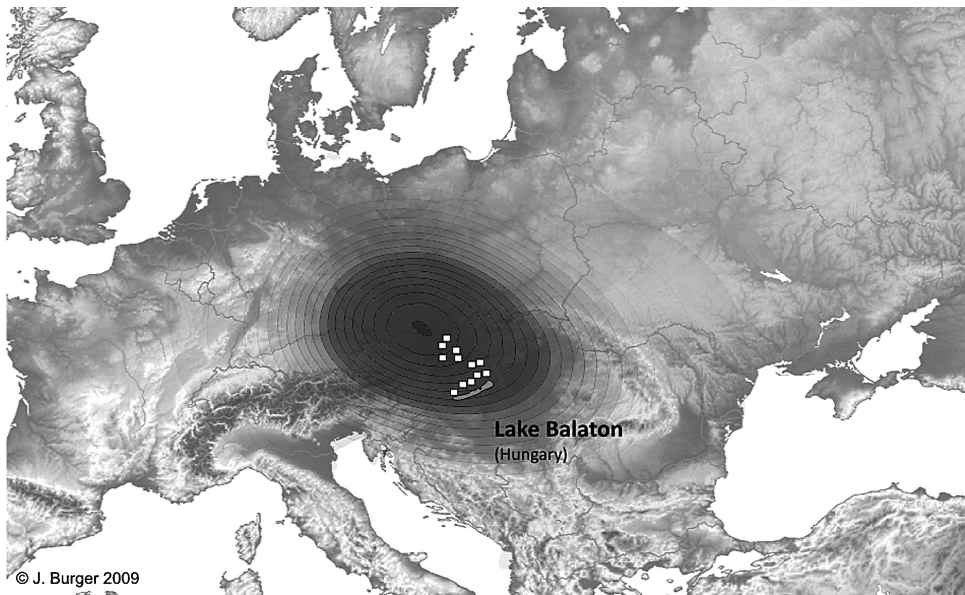

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Figure 15.2   Earliest known LBK sites (5,700–5,500 calBC; white squares) north of Lake Balaton after Pavuk (2005) and the inferred geographical origin of selection of lactase persistence, after Itan *et al.*, 2009 (about 4,310–6,730 calBC, concentric ellipses). (See Plate 15.2 for a colour version of this image)

a series of Neolithic expansions. It is possible that populations of the Starčevo-Körös-Cris complex were themselves the direct descendents of the earliest farmers to enter Europe, presumably from Western Anatolia or even from the western foothills of the secondary Neolithic core zone in Central Anatolia. If we consider the new picture that is now emerging for Central Europe on the basis of palaeogenetic data from domestic animals and humans, then an ultimate Anatolian (or even Near-Eastern) origin no longer seems as implausible as it once was.

We also need to consider the vexing question of hunter-farmer contacts in Europe. It is. however, necessary to point out that cultural and economic contacts may or may not lead to biological admixture. There is clear archaeological evidence of cultural exchange between hunter-gatherers and central Europe's first farmers, as witnessed, for example, in the Mesolithic silex artefacts found in LBK settlements (Gronenborn, 1999). Because the exchange of underlying silex technologies (e.g. sourcing, tool production methods) mainly involved hunting weapons, it is not unreasonable to assume that contact was primarily mediated by men. So far, there has been no palaeogenetic evidence of any admixture at all, if only because ancient Y-chromosome data has yet to be generated. The analysis of ancient Y-chromosome data is more challenging than for mtDNA, because of the reduced survival of nuclear DNA over time, but recent developments in sequencing technologies (Mardis, 2008) do offer a solution to this problem. However, where data does exist, there appears to be little if any exchange of mitochondria in the early centuries following the Neolithic transition in central Europe. Perhaps the most plausible scenario is that two distinct societies existed during this early Neolithic period, which interacted with each other culturally, but maintained female marriage restrictions and fundamentally differed in their economic and cultural attributes. There are numerous present-day parallels of this kind, where ethnic groups live side by side but have different economic systems. To give one example, in Mali the semi-nomadic Fulbe are cattle breeders and pastoralists, the Dogon are crop farmers but also keep some goats and sheep, and the Bozos are fishers of the Niger river. These three linguistically separated groups exchange the products of their work, for example the Fulbe exchange milk for millet with the Dogon but keep a strict marriage ban (Huysecom, personal communication). Although the precise nature of co-existence in Neolithic Europe will probably remain unknown, to infer the progressive degree of admixture between the two groups during the fourth and third millennia BC is an upcoming task of palaeogenetic research. Besides purely demographic phenomena and culturally defined barriers, selection of certain genetic traits is likely to have played an important role in the central European Neolithic transition. One such example is the rise of lactase persistence in Europe, a process on which we focus in the following section.

15.5 THE ROLE OF, AND ADAPTATIONS TO DAIRYING

Lactose is the main carbohydrate in milk and is a major energy source for most young mammals. The enzyme responsible for hydrolysis of lactose into glucose and galactose is lactase. Without this enzyme, mammals are unable to break down and thus utilize lactose. After the weaning period is over, lactase production usually declines. However, some humans continue to produce lactase throughout adult life, and are thus able to digest the lactose found in fresh milk; a trait which is called lactase persistence (LP). Genetic studies on modern

populations have shown that a common variant of the lactase gene (LCT) known as −13 910*T, is strongly associated with, and probably causative of LP in Europe (Enattah *et al.*, 2002) and appears to be one of the most strongly selected alleles in the human genome in Europeans in the last 30,000 years (Tishkoff *et al.*, 2007; Bersaglieri *et al.*, 2004; Coelho *et al.*, 2005). In Europe, clines are seen with LP found at frequencies of 10 to 30% in the south-eastern part of the continent, 50 to 60% in Central Europe, rising to 70 to 95% in north-western continental Europe and the British Isles. A similar trend is seen for the −13,910*T allele (Ingram *et al.*, 2009). Palaeogenetic typing of this variant site in 9 Meso- and Neolithic skeletons revealed an absence of the −13,910*T allele in all cases (Burger *et al.*, 2007). Although this is a relatively small sample, statistical analysis has demonstrated significantly lower frequencies of LP when compared to modern Europeans from the same region. This is consistent with previous studies, inferring high selection coefficients acting on this trait. It is unlikely that natural selection would have driven the −13,910*T allele to high frequencies without a supply of fresh milk and this ties the biological evolution of LP to the culture of dairying through a gene-culture co-evolution process. Clear evidence of milk production can be seen in South-east Europe as early as 6,200 calBC, using lipid analysis on pot sherds (Evershed *et al.*, 2008). In order to better understand the co-evolution of LP and dairying in Europe, Itan and colleagues (Itan *et al.*, 2009) developed a demic computer simulation model in order to examine how demographic and evolutionary parameters could have shaped both the modern distribution of LP in Europe and the timing of the arrival of farming at different locations throughout Europe. The study modelled the spread of dairying and non-dairying farmers into a Europe that was previously occupied by hunter-gatherers, under the plausible assumption that an LP-associated allele would only be selected in dairying farmers. Values for a number of different parameters must have shaped this process, including:

1. the extent of sporadic unidirectional migration;
2. the extent of gene flow between different cultural groups;
3. the extent of gene flow between neighbouring demes;
4. the extent to which people take up the culture of their neighbours;
5. the strength of selection favouring LP; and
6. the origin time and location of LP-dairying co-evolution.

By choosing random values for these parameters (within reasonable ranges), performing the simulation, and then comparing outcomes to observed data using Approximate Bayesian Computation (Beaumont, Zhang and Balding, 2002), the authors were able to identify parameter values that best explained the modern distribution of LP in Europe and the timing of the arrival of farming at different locations throughout Europe. Although the LP allele is most frequent in Northern Europe today, the simulations that best explained the observed data (on the distribution of LP and the arrival time of farming at different locations) required LP-dairying co-evolution to start in an area between the Carpathian Basin and Central Europe (Figure 15.2) between 6,260 and 8,680 years ago. The LP selection coefficient had inferred values between 0.0518 and 0.159 (in dairying farmers only). The inferred location and dates for the co-evolution of LP and dairying correspond well with the origins of the LBK culture in the Lake Balaton region (see Figure 15.2). This again is in accordance with Bramanti *et al.* (2009) who inferred a massive immigration of the LBK farmers from this region.

15.6 CONCLUSIONS

By integrating evidence from ancient and modern DNA, archaeozoology and archaeology, we now envisage the following plausible scenario: whereas in the early Neolithic there are already archaeometric indications of milk usage in West Anatolia (Evershed *et al.*, 2008) and the Balkans (Craig *et al.*, 2005), these early farmers (who imported their cattle ultimately from the Neolithic core zone in South-east Anatolia and the Northern Levant), were not able to consume significant quantities of fresh milk. Instead, they would have extracted the nutritional benefits of dairying by processing milk to make yoghurt, cheese and other products. In this process much or most of the lactose in the milk is converted to fatty acids, rendering the product consumable by lactase non-persistent individuals.

Starting in early Neolithic south-eastern Central Europe, LBK people began to settle in Central Europe from 5,500 calBC, without mixing significantly with the local hunters and gatherers. Although there are attested cultural contacts between the two groups, exchange of females is likely to have been limited, at least in the early stages. An initially rare −13,910*T allele began to rise in frequency as fresh milk became more readily available and the ability to drink it became more advantageous. We are not yet sure why LP provided such a big advantage at the time. The fact that it takes longer to ferment milk in cold climates may be a factor, but some buffering of the food supply, when compared to the boom-and-bust of seasonal crops, is most likely to have been involved, especially amongst pioneering farmers in uncharted territories. Significantly, the reduced mortality of post-weaning period lactase persistent children would have had a major demographic effect over the centuries. Already by the Middle Neolithic this co-evolutionary process had resulted in a specialized dairying economy something like we see in sites of the Rössen culture (Benecke, 1994). From 4,100 calBC, the Neolithic spread across the central low mountain regions to the North German lowlands and Northern Europe, would have been aided by the constancy of milk supply. Accordingly, a further increase in the frequency of the LP occurred, driven in part by selection and in part by the process of allele surfing (Edmonds, Lillie and Cavalli-Sforza, 2004; Klopfstein, Currat and Excoffier, 2006). Following further demographic expansion, the farmers that reached the north-western reaches of Europe were now predominately LP dairyers. We still don't know to which extent the regionally varying admixture with local hunter-gatherers influenced the LP frequency in this region.

Such scenarios are easy to envisage and eminently plausible. But to develop any real confidence in them, it is necessary to show by quantitative simulation modelling that the relevant processes fit well with ancient and modern DNA data, and also with the archaeological data. Only by this route, we believe, can the different data sources satisfactorily be integrated into a true understanding of the human past.

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