Transition to farming – transition to milk culture: a case study from Mala Triglavca, Slovenia

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ABSTRACT – In this paper, we discuss the transition to milk culture. While archaeological and biochemical data suggest that dairying was adopted in the Neolithic in Europe, archaeogenetic data show the absence of the allelic variant –13 910*T and very low lactase persistence in Neolithic populations in Europe. The Mala Triglavca case study shows that the Early Neolithic economy in the Caput Adriae region was mixed. It consisted of milk and processed milk, meat animal products, freshwater fish and various plants. The Vlaska group herders managed a broader spectrum of resources than exclusively ovicaprids, and were able to produce a wide range of low-lactose, storable products by fermenting milk.

IZVLEČEK – V članku razpravljamo o uporabi mleka in mlečni kulturi v prazgodovini. Medtem ko arheološki in biokemični podatki kažejo na razvoj mlekarstva v neolitiku v Evropi, arheogenetski govorijo o odsotnosti alela –13.910*T in laktozne persistence pri neolitskih populacijah v Evropi. Raziskave v Mali Triglavci kažejo, da je bilo gospodarstvo v zgodnjem neolitiku na območju Caput Adriae mešano, t.j. mlečno in mesno. Temeljilo je na uporabi mleka in mlečnih izdelkov, mesa prežvekovalcev in nепrežvekovalcev, sladkovodnih rib in različnih rastlin. Pastirji skupine Vlaska so v svoje gospodarstvo poleg vzreje drobnice vključili tudi druge vire. S pomočjo fermentacije so predelovali mleko v mlečne izdelke.

KEY WORDS – European Neolithic; demic diffusion; transition to farming; dairying; lactase persistence; lipids; Vlaska culture

Introduction

The beginning of dairy culture can be assumed to have occurred in the processes of the transition to farming, and the utilisation of lactic acid bacteria can be traced alongside the domestication of sheep, goat and cattle. In milking and milk processing, the lactococci and lactobacilli were manipulated to initiate the fermentation that converts milk into yogurt, buttermilk, butter and cheese. These have advantages in storing and transporting dairy products and making them available in times of low milk production on one hand, and making milk available as a nutritional source throughout the entire life of the individuals on the other. Biomolecular analyses of the lipids present in food which become absorbed and trapped in the pores of clay vessels show that milk was being used extensively by the 7th millennium BC in south-eastern Anatolia and around the Sea of Marmara. A millennium later, it was processed at some Early Neolithic sites in Southeast and Central Europe (Craig et al. 2005; Evershed et al. 2008). Parallel archaeogenetic studies hypothesised that a single mutation (–13 910*T) in the human genome which allow adults to consume fresh milk evolved within a group(s) of Neolithic pioneer stockbreeders among whom lactase persistence was rare, but who initially practised dairying in
Southeast Europe in the middle of 8th millennium BP and later migrated towards central and northern Europe to an area inhabited by foragers. They reached the northern Adriatic at c. 7400 BP (Gerbault et al. 2009; 2011; Burger, Thomas 2011; Gerbault 2012). However, the absence of the lactase gene in Neolithic populations in Europe shows that their lactase persistence was very low and ‘may have even been zero’ (Leonardi et al. 2012). Animal biomarkers observed in pottery in the Northern Adriatic suggest that dairy products were processed and stored at c. 6400 BP, and were associated with a mixed subsistence strategy based on meat (ruminant and non-ruminant origin), milk, plant and aquatic animals.

Dairying and lactase persistence

All humans have the lactase gene, but only children produce lactase in sufficient amounts to break down lactose, the main sugar in milk. Fresh milk is a toxin to adults without lactase, and often causes symptoms such as abdominal pain, bloating, flatulence and diarrhoea. Lactase is an enzyme produced in the digestive system of mammalian infants, but is dramatically reduced after the weaning period. The ability to digest lactose found in fresh milk is called lactase persistence. However, the correlation between lactase persistence and fresh milk consumption is not yet fully understood.

The lactase persistence trait is found in approx. 35% of adults in human populations in the world, but varies widely between and within continents. The frequencies of lactase-persistent individuals are generally high in Europe, Central Asia and India but almost zero in Southeast Asia (Ilan et al. 2010; Gerbault et al. 2011). In Europe, lactase persistence is at its highest frequency in the North, with a decreasing cline from the central and western (62–86%) to the southern and eastern regions (15–54%) (Gerbault et al. 2011). On the Indian sub-continent the frequency of lactase persistence is higher in the North-West than elsewhere; further East, the lactase persistence frequency is generally low. In Africa and the Middle East, the distribution is patchy, with some pastoral nomadic tribes having high frequencies (92%) of lactase persistence compared with neighbouring groups living in the same region (Tishkoff et al. 2007; Ingram et al. 2009; Gerbault et al. 2011).

A number of single nucleotide polymorphisms that allow lactase to be produced into adulthood have been found in different modern human populations worldwide. They are not located in the lactase gene (LCT), but in the intron of a neighbouring gene, MCM6, on chromosome 2. Several nucleotide changes in this region seem to affect lactase the gene promoter activity associated with lactase persistence (Gerbault et al. 2011). They have different geographic distributions within the modern populations. The derived allelic variant –13910*T of the first nucleotide cytosine to thymine transition C>T–13910 is associated with lactase persistence in Europe, Central Asia and India (Enattah et al. 2002; Ingram et al. 2007; Itan et al. 2009). This allele and associated selection for lactose tolerance seems to originate twice in ancestral populations (bearing haplotypes H) in regions north of the Caucasus and West of the Urals. The first origin is estimated at 12 000 to 5000 BP, and the second more recently at 3000 to 1400 years ago. It was suggested that the frequency gradient in modern populations shows that the allele migrated to the West (Enattah 2007.619–622).

Lactase persistence in Africa is linked to three single nucleotide polymorphisms, C–14 010, G–13 915 and G–13 907, close to the lactase gene (Tishkoff et al. 2007). They are linked to different ethnic groups...
with divergent haplotype backgrounds and geographic regions. However, some questions still remain unanswered. The Hadza people in Tanzania show a high level of lactase persistence despite having nothing to do with herding. The possible explanation is that, although they are now mainly hunter-gatherers, their ancestors might have been pastoralists. European –13 910*T and East African G–13 907 LP alleles are very near to each other and probably share the same ancestral allele that “might have arisen because of a common domestication event of the cattle, whereas the C–3712 and G–13 915 allele in Arabia most likely arose due to the separate domestication event of camels” (Enattah et al. 2008:70). The origin of the African allele G/C–14 010 is estimated between c. 6800–2700 BP (Tishkoff et al. 2007:36).

Lactase persistence is one of the leading examples of natural selection in humans and also one of the first clear examples of the polymorphism of a regulatory gene in the human genome (Ingram et al. 2009). A single gene was involved with different mutations in different parts of the world, but with similar effects. The lactase persistence has been mainly identified in pastoralist populations and, as fresh milk and milk products are the only known naturally occurring sources of lactose, it is therefore unlikely that this trait would be selected without a supply of fresh milk (Gerbault et al. 2011). Why this trait was so strongly selected is still widely discussed.

Several scenarios relating to the ‘selection hypotheses on lactase persistence’ and to ‘the advantage of being lactase persistent’ have been discussed recently. The first and most widely accepted ‘gene – culture coevolution’ or ‘culture historical’ hypothesis proposes that lactase persistence was selected among populations that consumed milk over generations and adopted animal breeding and dairying, thereby increasing the dependence of adults on milk. In opposition, the second, the ‘reverse cause hypothesis’, suggests that dairying was adapted by populations that were already lactase persistent. A mutation associated with lactase persistence within small human groups could have grown in frequency through genetic drift before milk was introduced into subsistence. The third, the ‘calcium assimilation hypothesis’, suggests that in high-latitude environments where lower sunlight produces less vitamin D (important for the absorption of calcium in bones) lactose in fresh milk promotes the uptake of calcium present in milk. In contrast to hunter-gatherers who had a vitamin D rich diet abundant in marine food, early agriculturalist might have had problems with vitamin D deficiency, and drinking milk could have been an advantage for lactase-persistent farmers (i.e. in the Neolithic). The fourth, the ‘arid climate hypothesis’, suggests that in regions where water was scarce, milk could be an uncontaminated source of fluid used by pastoralists. While lactase non-persistent individuals were at risk from diarrhoea and the dehydrating effects of drinking fresh milk, the selection may have been strong in lactase-persistent individuals (for a detailed overview, see Aoki 1986; 2001; Holden, Mace 1997; Bloom, Sherman 2005; Ilan et al. 2010; Gerbault et al. 2011; Liebert 2012).

However, archaeological and archaeogenetic data suggest that dairying in Europe was adopted before lactase persistence became frequent. The absence of the allelic variant –13 910*T shows that lactase persistence in Neolithic populations in Europe was very low and ‘may have even been zero’ (Leonardi et al. 2012:93). On the contrary, the stable isotope analyses of dairy fats in pottery suggest that milking, milk consumption and processing were widely adopted in the Neolithic. Pastoralism was thus adopted before lactase persistence arose or became frequent. We may assume, therefore, that under normal circumstances lactase persistence is not necessarily to be under very strong selection in this population and fits with the hypothesis that dairying and milk consumption emerged before genetic adaptation. Strong selective pressures may have been episodic and occurred only under certain extreme circumstances, such as drought, epidemic or famine.

The transition to milk culture

We may assume that animal domestication brought milk into the diet, and that domestic animals were a more stable seasonal resource, which could become an alternative to hunter-gatherers’ system of the seasonal exploitation of a broad spectrum of animal resources. Milk is a good source of calories, specifically an important source of protein and fat, and must have increased the quality of the diet. “The milk production of a prehistoric cow has been estimated to range between 400 and 600kg per weaning period. Even when the milk necessary for the raising of the calves is subtracted, some 150–250kg remains. This is almost equivalent to the calorie gain from the meat of a whole cow. Hence, over the years, milking may have resulted in a greater energy persistence would have been selected in regions where the disease was frequent. Hence, over the years, milking may have resulted in a greater
energy yield than the use of cattle for meat” (Gerbault et al. 2011.865–866). Dairying was especially important for children and adolescents as it prolongs the beneficial effects of milk (proteins, fats, but also calcium supply) long after weaning (Vigne 2008.200; Panesar 2011).

Jean-Denis Vigne (2008) suggests that ungulate domestication (e.g., sheep, goat and cattle) in the Near East was part of the diet transition that ‘correlates to deep changes in food supply’. It was not because of a better meat supply, but the introduction of milk and milk products that have brought significant modifications to the human diet. Indeed, herd management in the initial Near Eastern Neolithic shows sophisticated herding practices for milk. It implies that large numbers of ovicaprid and bovine female animals were kept; techniques for separately herding young and adults, with particular attention to lambs; and an increase in infant and old-age culling in mortality profiles (Helmer et al. 2007; Vigne, Helmer 2007; Vigne et al. 2011.S266; Çakırlar 2012a; 2012b).

It is worth noting that modern and ancient cattle mtDNA sequences do not support the currently accepted hypothesis of a single Neolithic origin in the Near East. The processes of livestock domestication and diffusion were certainly more complex than previously suggested, and genetic data provide some evidence in favour of the hypothesis that European cattle had multiple origins. Breeds domesticated in the Near East and introduced to Europe during the Neolithic diffusion probably interbred, at least in some regions, with local wild animals and with African cattle introduced by maritime routes. It is possible that pastoralist societies in Southern and Northern Europe used different breeding techniques, with the latter more concerned with herd protection (Beja-Pereira et al. 2006; see also Edwards et al. 2011).

In addition, stable isotope analyses of dairy fats in pots show evidence of dairy production in southwest Asia as early as 8500 BP. The apparent intensification of dairy processing in northwest Anatolia was recognised as an early centre for milk processing, with cow’s milk as the main source of dairy products in this region (Evershed et al. 2008; Thissen et al. 2010; for comments see Çakırlar 2012a). This region had a central position in dispersals of Neolithic subsistence economies into Europe (Brami, Heyd 2011; Özdoğan 2011).

On the other hand, Albano Beja-Pereira et al. (2003) suggest that the strong geographic correlation between cattle milk gene diversity, human lactose tolerance and the distribution of the earliest European cattle pastoralists began in North-Central Europe and led to genetic co-evolution between humans and domestic animals, *i.e.* the ability of adults to consume milk and the ability of dairy cattle to give high milk yields (Beja-Pereira et al. 2003). Allele -13 910*Tcline indeed shows frequencies decreasing from North to South. In Scandinavia, where dairying arrived late, almost all the sampled individuals are lactase-persistent, with frequencies ranging between 96% and 89%. In Southern Europe, it ranges between 15% and 54% (Gerbault et al. 2011.864).

The presence of abundant milk fat and specialised vessels used to separate fat-rich milk curds from lactose-containing whey indeed provide direct chemical evidence of milk processing in Northern Europe in the Early Neolithic LBK complex. The pottery assemblage is dated to c. 5200 and 4900–4800 calBC (Salque et al. 2012). Oliver Craig et al. (2005) provided much earlier data for milk processing in the Early Neolithic in Southeast Europe. Degraded ruminant fatty acid in pottery in the Starčevo-Criş (5950–5500 calBC) and Köros culture (5800–5700 calBC) suggest milk products and milk processing, *i.e.* the heating of milk.

It should be noted that lactose is progressively reduced by milk processing. The lactose content of fresh milk ranges between 4.42–5.15 g/g% in cattle, 4.66–4.82 g/g% in goats and 4.57–5.40 g/g% in sheep. It can be reduced to 50–60% by bacterial fermentation. Some processed milk products (such as cheese and butter) have very low lactose content, ranging from 0–3.7 g/g% (Nagy 2011.267; Liebert 2012.77).

Milk is extremely perishable and many methods have been developed to preserve it; fermentation was the earliest method. Inoculating fresh milk with the appropriate bacteria can ferment milk at temperatures that favour bacterial growth. As the bacteria grow, they convert milk sugar or lactose to lactic acid. The lowered pH caused by lactic acid preserves the milk by preventing the growth of pathogenic bacteria which do not grow well in acidic conditions. The lactic acid bacteria (*e.g.*, *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus caucasicus and Lactococcus lactis*) thus turn milk into yogurt, kefir, buttermilk and cheese. They make milk available as a nutritional source throughout the entire life of individuals on the one hand, and allow advantages in storing and transporting dairy.
products and making them available in periods of low milk production on the other.

**Lactase persistence and demic diffusion migratory model**

Pascale Gerbault *et al.* (2009; 2011; 2012) and Yuval Itan *et al.* (2009; 2010) intensively studied the evolutionary processes that shaped the European lactase persistence patterns in modern populations. They ran computer simulations to test different selection hypotheses on lactase persistence in relation to demic diffusion and culture diffusion models. Their results are contrasting. Computer simulations showed that high lactase persistence frequencies observed in Northern and Western Europe can be explained by selective pressure, possibly increasing with latitude in a way that is highly compatible with the calcium assimilation hypothesis combined with the effect of demographic expansion (i.e. population growth) during the Neolithic transition. The much lower frequencies in Southeast Europe can be explained by genetic drift if this mutation was carried by Near-eastern pioneers. Keeping in mind that the demic diffusion model is based on the decreasing southeast-northwest cline of frequencies for selected Y-chromosome markers, indicating the movement of Neolithic men with Levantine genetic ancestry across Europe, it is important to note that the allelic variant -13 910*T cline travels in the opposite direction. However, computer modelling suggests that the centre of distribution of an allele can be far removed from its location of origin in the direction of population expansion, moving at the front of the demic diffusion. This process is called ‘allele surfing’ and is thought to have occurred with the spread of farmers in Europe (Gerbault *et al.* 2009.3, 7–8, Fig. 1; 2011; see also Gerbault 2012.179–198, Fig. 4) thus hypothesised that strong selection for lactase persistence runs within the ‘niche construction’ at the front of the demic diffusion, where local environmental condition and subsistence strategies led to population increase and concentration on milk resources. The initial selection was embedded in Southeast Europe at 8518±66 BP (7592–7528 calBC), and the first lactase persistent farmers and domesticates arrived in Central Europe and the Northern Adriatic a millennium later, at 7416±101 BP (6418–6213 calBC). The latter 14C date was contextualised in the Edera/Stena rock shelter in the Trieste Karst and is linked to the Early Neolithic Vlaška culture (Pinhasi *et al.* 2005. Supporting information, Tab. 1) (Fig. 1).

Nevertheless, Itan *et al.* (2009; 2010; see also Burger, Thomas 2011; Leonardi *et al.* 2012) suggest

**Fig. 1. Map of European and Near-Eastern populations used for the computer simulation test of gene-culture coevolution and calcium assimilation hypotheses of lactase gene selection. It implies that positively selected lactase gene (with frequencies that increase with latitude) was carried over Europe at the front of the Neolithic demic diffusion (from Gerbault *et al.* 2009. Fig. 1).**

1 For discussion that farming niche is not necessary an effective strategy for achieving demographic and economic stability see Rowley-Conwy and Layton (2011).

2 Calibrated with Oxcal 4.2 programme at 1σ.
that the −13 910*T allele first underwent selection in a relatively short period among dairy farmers in the northern Balkans in the Starčevo and Körös cultures. It was then dispersed by demic diffusion to Central and Western Europe in the area of Linear Pottery culture at ‘around 6256–8683 years BP’ (Ilan et al. 2009; see also Ilan et al. 2010).

However, both scenarios, the demic diffusion of lactase-persistent farmers across Europe and the evolution of lactase persistence in Central Europe in the Neolithic, seem to be unrealistic. The archaeogenetic analysis of Neolithic skeletons suggests that ‘lactase persistence frequency was significantly lower in early Neolithic Europeans than it is today, and may have been zero’ (Leonardi et al. 2012; 93; see also Burger, Thomas 2011). Indeed, the analysis revealed an absence of the −13 910*T allele in Central Europe, in the Western Mediterranean and the Baltic in Mesolithic and Neolithic populations (Burger et al. 2007; Burger, Thomas 2011; Lacan et al. 2011; Linderholm 2011; Nagy et al. 2011). The only exceptions are two post-Neolithic individuals in the Basque Country on the Iberian Peninsula (Plantinga et al. 2012).

Early farming and milking in the Caput Adriac (Northern Adriatic)

The distribution of the first farming communities in the Eastern Adriatic is traditionally associated with the ‘Impresso Cardium’ (i.e. impressed) pottery dispersal. It was also used as an indicator of the spread of farming across the region. Stašo Forenbaher and Preston Miracle (2005; 2006) introduced a two-phase model suggesting that impressed ware originated in coastal Northern Greece and spread with immigration from South to North along the Adriatic coast. The process included immigrant farmers that made exploratory visits and set up short-term seasonal camps at caves and open-air sites along the coastal strip of southern Dalmatia (i.e. pioneer colonisation), followed by a village settlement that spread slowly towards the Northern Adriatic in areas with fertile soils (i.e. consolidation phase).

The northern boundary of ‘Impresso Cardium’ pottery through the Eastern Adriatic is positioned in southern Istria, as no early farming sites with impressed ware are known from northern Istria and the coastal fringe of the Trieste Bay. Indeed, in most sites across the boundary, the earliest Neolithic is represented by Vlaška pottery (Barfield 1972). These pottery assemblages resemble those from the Middle Neolithic Danilo culture in Dalmatia, and it has been hypothesised that the region was not colonised before the Middle Neolithic (Forenbaher, Miracle 2006; Forenbaher, Kaiser 2006; Biagi, Spataro 2001; Biagi 2003). The Vlaška and Danilo pottery assemblages have similar characteristics in their shapes, decoration techniques and motifs. However, the main difference between them is the absence of painted pottery in Vlaška assemblages.

Nevertheless, we may assume that the Vlaška group does not represent the initial Neolithic in the region. Materialities in stratigraphically super-positioned layers 2a and 3a at the Edera/Stena ka rock shelter show that the first can be recognised as the Vlaška group, but the latter contained plain pottery of local and non-local manufacture, along with the bones of domestic (i.e. caprines, cattle and pig) and wild animals, shells of marine molluscs and lithics that includes trapezes and microburins. It was recognised as a Late Castelnovian hunter-gatherer complex and dated to 6700 ± 130 BP (5700–5515 calBC) (Biagi, Spataro 2001.35).

The beginning of the Early Neolithic in the Eastern Adriatic appears to be embedded in the time span between 6048–5988 calBC in the North (Vela spilja, Mali Lošinj island), 5985–5843 calBC in the central region (Pokrovnik in Dalmatia), and between 5986–5903 calBC (Spila Nakovana on Pelješac peninsula) and 5989–5767 (Vela spilja on Korčula Island) in the South. The available 14C evidences in the Northern Adriatic show that the Istrian peninsula and Karst Plateau above Trieste Bay remained outside this range. It is postulated that the Neolithic was established here at c. 5600 calBC and that it was associated with the end of “Impressed Ware and the appearance of assemblages with only undecorated pottery” (i.e. Vlaška-Danilo pottery) in the Middle Neolithic (Forenbaher et al. 2013;599) (Fig. 2).

Interestingly, Mesolithic sites are known in this area (Komšo 2006), but none is securely dated to the pe-
period after 6000 calBC. On the other hand, radiocarbon sequences from sites in this region show a temporal gap between the latest Mesolithic and earliest Neolithic occupations that varied in duration and were not synchronous among the sites, although there is an evident continuity of occupation over the wider region. Various hypotheses have already been proposed to account for the temporal discontinuity, but it remains unresolved (for discussions, see Biagi, Spataro 2001; Biagi 2003; Forenbaher, Miracle 2006.497-504; Mlekuž et al. 2008; Berger, Guiattaine 2009; Bonsall et al. 2013).

How the Neolithic Vlaška group herders managed ovicaprids

The question of how the Neolithic Vlaška group herders managed herds has been addressed already. Most authors agree that Vlaška group herders were involved in some form of transhumant or nomadic pastoralism, with seasonal occupation of cave sites (Miracle, Pugsley 2006; Mlekuž 2005). However, contradictory scenarios have been proposed based on the interpretation of kill-off curves (see Rowley-Conwy 2013.163–174).

It is worth remembering that Sebastian Payne (1973) proposed – on the basis of his ethnoarchaeological research among Turkish pastoralists – a middle range theory, which links flock management strategies to kill-off curves. It is based on the assumption that an optimisation of animal products can be obtained by manipulating the sex and age structure of the herd. Ideal dairying and meat models differ in the age when males are culled. In the ideal dairying model, most animals younger than two months are culled in order to reduce competition for milk with people. With an optimal meat strategy, most animals are culled after one to three years, as they achieve their maximum weight.

The interpretation of kill-off curves is complicated by a strong preservation bias against neonates and young animals on the one hand and a high natural mortality of neonates and young animals on the other. But the main problem behind the use of idealised curves is the assumption that people in the past behaved optimally. Ethnographic evidence suggests that within household-based economies, animals are used for a variety of animal products. Specialised and optimised exploitation of animal products emerges from the demands of a market-based economy. Thus, a correspondence to the ideal ‘dairying’ model would indicate specialised production geared towards exchange (Halstead 1996.25; 1998).

Two new models for detecting animal exploitation for meat and milk have been proposed recently (Vigne, Helmer 2007; Vigne 2008). They suggest that caprine and cattle culling profiles in the Near East and the Mediterranean show that the exploitation of cattle, sheep and goats was aimed at milk production and not only meat from the initial Neolithic onwards. While small herds of goats were exploited mainly for milk, larger sheep herds were also for meat production. However, no curve resembles ideal strategies based on either meat or milk.

Vlaška group bone assemblages are comprised predominately of sheep and goat bones (around 60% of sheep and goats and less than 10% of cattle; see Mlekuž 2005). Milk yields from small stock are generally much lower than those of cows (goats, which have up to 100% higher yields than sheep, typically produce around 125kg of milk per lactation); however, they have a very high rate of increase (up to ten times compared to cattle). This makes them especially suitable for the accumulation of large herds (see Ingold 1980; Dahl, Hjort 1976; Mlekuž 2005).
Kill-off curves from the North Adriatic region (Miracle, Förenbächer 2005; Miracle, Pugsley 2006. 319–335, Fig. 7.27) were interpreted as a result of the management of herds aimed at harvesting dairying products. Dimitrij Mlekuž (2005; 2006), on the contrary, suggested that kill-off curves demonstrate a relatively simple, non-optimised economy aimed primarily at the domestic consumption of meat, not strategies aimed at maximising dairy products (Fig. 3). However, this does not exclude small-scale dairying of sheep and goats. Since goats are more effective milk producers than sheep (Dahl, Hjort 1976. 210), one would assume that goats were milked (Rowley-Conwy 2000). Goats are present after the appearance of small stock in Caput Adriae. However, their proportion compared to sheep is relatively low, around 20%, rendering their role in small-scale dairying invisible in the crude resolution of survivorship curves.

Molecular and isotope evidence of dairying and food processing in the Caput Adriae: Mala Triglavca case study

We present the results of the organic residues analyses of pottery deposited in a stratified deposit at the Mala Triglavca rockshelter. The site is located on the Dinaric Karst in south-western Slovenia, 15km from the Northern Adriatic coast (Fig. 2). The site is still being excavated, but the pilot molecular and isotope analyses have already shown well-preserved lipid residues and the presence of dairy products (Söberl et al. 2008). The AMS $^{14}$C dates show a long sequence of human agency from the 8th to the 3rd millennium calBC, combined with post-depositional disturbances of natural and geomorphological processes (Mlekuž et al. 2008) (Fig. 4).

The evidence from the current excavations and associated soil/sediment analyses show that in the central part of the cave a well-defined stratigraphic sequence can be established, despite post-depositional modifications by soil-forming processes. There is, however, evidence of post-depositional disturbances of cave sediments by human agency and geological/geomorphological processes such as rotational slumps, as verified by the presence of distinct shear planes near the cave wall.

The sequences in the central part of the rock shelter consist of a series of thin white powdery layers interleaved with darker layers. This rhythmic depositional sequence can be interpreted as a series of occupation levels. Each occupation started with the preparation of a new floor, sometimes by using large platy stones to cover the previous heap of ash. Irregularities and depressions were filled with stones, so that the floors in the rock shelter were kept horizontal. At the end of occupation, organic debris was cleaned and the collected material heaped and burned; the result is a new heap of ash.

Nevertheless, there are some differences between occupational levels. The quantity of the deposited material varies vastly between levels. Some occupation levels are very simple, while others are quite elaborate, with well-paved surfaces made from large platy stones. In some levels, we have evidence of vertical elements that were part of the occupation floor. Thus, we have found several elaborately built circular stone structures. We have identified 25 occupation episodes so far, embedded in the time span of c. 5800 calBC (level 19) and c. 3500 calBC (level 1) so far (Tab. 1; Fig. 4).

The Neolithic and Eneolithic pottery assemblage of occupational levels 1 to 23 is comprised mostly of various types of bowls, but beakers, dishes and pots are also present, and most of these have a simple outline (Tab. 1; Fig. 5). According to their shape and decoration, most of the vessels from occupational level 6 onwards can be linked to the Vlaška pottery group as described by Lawrence Barfield (1972). The oldest pottery fragments appear as early as 5616–5525 calBC and have the same technological characteristics as the typical Vlaška pottery at the site, i.e. with local clays prepared with added cru-
shed calcite (Žibrat Gašparič 2004). On the other hand, these oldest pottery fragments were mostly polished and undecorated, i.e. they do not exhibit typical Vlaška decorations.

We tested the hypothesis that dairying was an integral part of a mixed subsistence strategy from the initial Neolithic onwards by examining a range of pottery from the site. For the lipid analysis, we sampled 29 vessels from Mala Triglavca from contexts ranging from 5467–5356 to 4338–4261 calBC (Fig. 5; Tabs. 1 and 2). The samples were chosen according to their stratigraphic position, typology and decoration characteristics. The majority of samples (83.3%) are various types of bowls with incised ornaments or appliqués typical of the Vlaška group. The remaining samples were two pots and two deep dishes. The samples are all made of fabrics with added calcite grains as temper and are mostly fine-grained (75%), very fine-grained (20.8%); only one sample had a coarser-grained temper. The surfaces of the vessels were mostly burnished (54.2%) and polished (29.3%); smoothing is less common (16.7%). The surface colour of the vessels is predominantly light brown

| Occupation level | Structures | Predominant types of food | δ¹³Cbulk ±0.2 (‰) | Radiocarbon date | Lab code |
|------------------|------------|---------------------------|--------------------|-----------------|----------|
| 1                |            |                           | 3690±40*           | Poz–15343       |          |
| 2                |            |                           | 5530±60             | Beta–23604      |          |
| 3                |            | dairy (87MT)***; ruminant adipose fat (MaTr151) | MaTr151: –28,0     | 6960±170**       | Poz–48531 |
| 4                |            | dairy (MaTr130, MaTr137); ruminant adipose fat (MaTr112, MaTr115, MaTr116) | MaTr130: –27,8; MaTr137: –27,0; MaTr112: –25,6; MaTr115: –26,4; MaTr116: –24,9 | 6390±40* | Poz–48530 |
| 5                |            | freshwater fish (MaTr470) | –25,7              |                 |          |
| 6                |            | dairy (93MT)***; ruminant adipose fat (MaTr147) | MaTr147: –26,6      | 6320±40*         | Poz–21395 |
| 7                |            | ruminant adipose fat (MaTr159); mixed, plant (MaTr145) | MaTr159: –26,0; MaTr145: –26,2 | 5660±40**       | Poz–48543 |
| 8                |            | postholes dairy (75MT, 161MT)***; mixed (MaTr53); mixed, plant (MaTr107) | MaTr53: –25,4; MaTr107: –26,3 | 6340±40* | Poz–48539 |
| 9                |            | circular structure dairy (MaTr174); ruminant adipose fat (MaTr173, MaTr600) | MaTr174: –26,5; MaTr173: –25,9; MaTr600: –26,0 | 6940±40** | Poz–48542 |
| 10               |            | mixed (159MT)***          |                    | 6400±40          | Poz–48541 |
| 11               |            |                           |                    | 6410±40          | Poz–48538 |
| 12               |            | circular structure dairy (MaTr602); freshwater fish (MaTr599); mixed, plant (MaTr606) | MaTr602: –26,3; MaTr599: –25,4; MaTr606: –23,0 | 6620±40* | Poz–48540 |
| 13               |            |                           |                    |                 |          |
| 14               |            |                           |                    |                 |          |
| 15               |            |                           |                    |                 |          |
| 16               |            |                           |                    |                 |          |
| 17               |            |                           |                    |                 |          |
| 18               |            |                           |                    |                 |          |
| 19               |            |                           |                    |                 |          |
| 20               |            |                           |                    |                 |          |
| 21               |            |                           |                    |                 |          |
| 22               |            |                           |                    |                 |          |
| 23               |            |                           |                    |                 |          |
| 24               |            |                           |                    |                 |          |

* Date from Leben’s excavations
** Stratigraphic/¹⁴C inconsistency
*** Stratigraphic position based in correlation with Leben’s excavated levels.

Tab. 1. The composite table presenting the occupational levels at Mala Triglavca, the AMS ¹⁴C dates and the lipid residues from pottery.
Various types of decoration were present in 45.8% of the samples, but most of the vessels are undecorated (54.2%). The decoration techniques included incisions, appliqués and rare impressions, or a combination of these techniques. The motifs include: a band of hatched pendant triangles (samples MaTr112, MaTr470; Fig. 5.3), which is the most typical motifs of the Vlaška group (Barfield 1972.202, Taf. 50.8–11); a curvilinear garland motif (sample MaTr115; Fig. 5.2) and a plant motif (sample MaTr130; Fig. 5.4). The plant motif is rare in the Caput Adriae region, but appears mostly on typical Vlaška bowls (Barfield 1972.202, Taf. 50.15). Similar decorations are known from the Trieste Karst area, such as on a bowl from layer 5 at Grotta degli Zingari/Ciganska jama (Gilli, Montagnari Kokelj 1996.75, Fig. 12.48), which may have affinities with Linear Pottery culture (Barfield 1999.30).

**Material and methods**

We used all the 29 pottery fragments for chemical study lipid distribution including fatty acids, stable isotope composition (bulk δ13C or δ15N, and δ13C of individual fatty acids) and the di- and triacylglycerols (DAGs and TAGs) distribution of organic residues (Tab. 2). The sherds were surface cleaned to
| Lab. sample no. | Occupation period | Description | δ¹³Cbulk ±0.2 (%) | δ¹⁵Nbulk ±0.3 (%) | Lipid conc. (µg g⁻¹) | δ¹³C₁₆₋₂₀ ±0.3 (%) | δ¹³C₁₈₋₂₀ ±0.3 (%) | Δ³¹C (%) | C₆₀/₇ₐ/₈₀ | Mono-, di- and triacylglycerols | Fatty acids (FA) | Other lipids | Predominant commodity type |
|----------------|------------------|-------------|------------------|------------------|---------------------|------------------|------------------|-------------|-----------------|------------------------------|-----------------|-----------------|-----------------------------|
| MaTr53         | 14 bowl          | -25.4       | 4.6              | 8.62             | -33.1              | -32.6            | 0.50             | 2.6         | n/d             | C₁₁₀, C₁₈₁₁, C₁₈₀, C₂₀₀       | WE              | mixed           |                             |
| MaTr100        | / bowl           | -26.4       | 5.4              | 22.1             | -29.6              | -30.4            | -0.8             | 1.7         | n/d             | C₁₁₀, C₁₈₁₀, C₁₈₀, C₂₁₀       | n/d              | mixed           |                             |
| MaTr107        | 14 dish          | -26.3       | n/d              | 42.1             | -33.4              | -34.2            | -0.8             | 1.9         | n/d             | C₁₁₀, C₁₅₀₁₁, C₁₈₁₀, C₂₀₀     | ALK, WE         | mixed, plant     |                             |
| MaTr109        | 13 pot           | n/d         | 6.56             | n/d              | n/d                | n/d              | n/d              | n/d         | n/d             |                              | n/d              | n/d              |                             |
| MaTr112        | 10 bowl          | -25.6       | 51.7             | -29.7            | -31.4              | -1.7             | 1.7              | n/d         | DAG             | C₁₁₀, C₁₈₁₁, C₁₈₀          | CH               | ruminant adipose fat |                             |
| MaTr115        | 10 bowl          | -26.4       | 9.4              | 60.5             | -31.8              | -32.9            | -1.1             | 1.8         | TAG             | C₁₁₀, C₁₅₀₁₁, C₁₈₁₀, C₂₀₀     | ALK              | ruminant adipose fat |                             |
| MaTr116        | 10 bowl          | -24.9       | n/d              | 38.7             | -31.6              | -33.4            | -1.8             | 1.7         | n/d             |                              | n/d              | ruminant adipose fat |                             |
| MaTr125        | 10 bowl          | n/d         | 6.41             | n/d              | n/d                | n/d              | n/d              | n/d         | n/d             |                              | n/d              | n/d              |                             |
| MaTr130        | 10 bowl          | -27.8       | 5.4              | 19.1             | -29.6              | -33.1            | -3.5             | 1.5         | DAG, TAG        | C₁₁₀, C₁₈₀             | CH               | dairy            |                             |
| MaTr145        | 12 bowl          | -28.0       | 2.5              | 14.2             | -29.0              | -30.8            | 1.80            | 2.7         | ALK, WE         | C₁₁₀, C₁₇₀₁₁, C₁₈₀          | ALK              | mixed, plant     |                             |
| MaTr147        | 12 bowl          | -26.6       | 7.8              | 8.02             | -30.1              | -31.8            | -1.70            | 2.1         | n/d             | C₁₁₀, C₁₇₀₁₁, C₁₈₀          | ALK              | ruminant adipose fat |                             |
| MaTr151        | 9 bowl           | -28.0       | 2.5              | 14.2             | -29.0              | -30.8            | 1.80            | 2.7         | DAG, TAG        | C₁₁₀, C₁₇₀₁₁, C₁₈₀          | ALK, WE          | ruminant adipose fat |                             |
| MaTr159        | 13 bowl          | -26.0       | 8.2              | 12.6             | -28.1              | -30.6            | -2.50            | 2.5         | n/d             | C₁₁₀, C₁₇₀₁₁, C₁₈₀          | ALK, OH, WE      | ruminant adipose fat |                             |
| MaTr160        | / dish           | n/d         | 3.08             | n/d              | n/d                | n/d              | n/d              | n/d         | n/d             |                              | n/d              | n/d              |                             |
| MaTr173        | 15 pot           | -25.9       | n/d              | 17.6             | -31.3              | -32.9            | 1.5              | n/d         | n/d             | C₁₁₀, C₁₈₁₁, C₁₈₀          | n/d              | ruminant adipose fat |                             |
| MaTr174        | 15 bowl          | -26.3       | 6.3              | 20.2             | -30.1              | -34.5            | -4.40            | 3.3         | n/d             | C₁₁₀, C₁₇₀₁₁, C₁₈₀          | n/d              | dairy            |                             |
| MaTr180        | 6 bowl           | -25.7       | 10.3             | 11.0             | -31.4              | -29.0            | 2.40             | 2.5         | DAG             | C₁₁₀, C₁₇₀₁₁, C₁₈₀          | ALK, WE          | freshwater fish |                             |
| MaTr183        | 3 bowl           | n/d         | 7.37             | n/d              | n/d                | n/d              | n/d              | n/d         | n/d             |                              | n/d              | n/d              |                             |
| MaTr199        | 19 bowl          | -25.4       | 8.4              | 14.5             | -32.1              | -30.1            | 2.00             | 4.8         | n/d             | C₁₁₀, C₁₄₀₁₁, C₁₈₀          | ALK, WE          | freshwater fish |                             |
| MaTr200        | 15 bowl          | -26.0       | n/d              | 46.7             | -31.2              | -32.5            | -1.3             | 1.7         | n/d             | C₁₁₀, C₁₈₁₁, C₁₈₀          | n/d              | ruminant adipose fat |                             |
| MaTr202        | 19 bowl          | -26.3       | 9.7              | 11.5             | -30.7              | -34.1            | -3.40            | 2.7         | MAG, DAG, TAG  | C₁₁₀, C₁₄₀₁₁, C₁₈₀          | CH, ALK          | dairy            |                             |
| MaTr205        | 19 bowl          | n/d         | 3.33             | n/d              | n/d                | n/d              | n/d              | n/d         | n/d             |                              | n/d              | n/d              |                             |
| MaTr206        | 19 bowl          | -23.0       | 0.1              | 14.4             | -30.9              | -30.7            | 0.20             | 3.6         | n/d             | C₁₁₀, C₁₄₀₁₁, C₁₈₀          | ALK              | mixed, plant     |                             |
| 79MT¹          | 14-15ª bowl      | n/d         | 30.0             | -27.7            | -31.1              | -3.3             | n/d              | MAG, DAG, TAG | C₁₁₀, C₁₄₀₁₁, C₁₇₀₁₁, C₁₈₁₀, C₂₀₀ | CH, ALK, OH     | dairy            |                             |
| 87MT¹          | 8-10ª beaker     | n/d         | 20.9             | -27.3            | -31.9              | -4.6             | 1.6              | n/d         | C₁₁₀, C₁₇₀₁₁, C₁₈₁₀, C₂₀₀     | CH, ALK          | dairy            |                             |
| 156MT¹         | 15-17ª pot       | n/d         | 10.0             | n/d              | n/d                | n/d              | n/d              | n/d         | n/d             |                              | n/d              | n/d              |                             |
| 159MT¹         | 15-17ª bowl      | n/d         | 11.5             | -28.8            | -28.6              | 0.2              | n/d              | MAG, DAG, TAG | C₁₁₀, C₁₅₀₁₁, C₁₇₀₁₁, C₁₈₁₀, C₂₀₀ | CH               | mixed            |                             |
| 161MT¹         | 14-15ª bowl      | n/d         | 42.4             | -28.1            | -32.2              | -5.1             | n/d              | MAG, DAG     | C₁₁₀, C₁₅₀₁₁, C₁₇₀₁₁, C₁₈₁₀, C₂₀₀ | n/d              | dairy            |                             |

¹ Samples re-analysed, see Šoberl et al. 2008.257, Tab. 1; ² Stratigraphic position based in correlation with Leben's excavated levels.

### Tab. 2. A summary of lipid residues detected in pottery vessels in Mala Triglavca. Key: MAG – monacylglycerols; DAG – diacylglycerols; TAG – triacylglycerols; ALK – n-alkanes; OH – n-alcohols; WE – wax esters; CH – cholesterol; n/d – not detected.
remove any exogenous lipids. The sub-samples were then ground to a fine powder. In addition, fat samples of modern cattle, sheep and cow that have been fed exclusively on C3 forage grasses on the Karst plateau were analysed in order to test the origin of the fat in the archaeological ceramics (Tab. 3).

First, powder samples were analysed by elemental analysis isotope ratio mass spectrometry (IRMS) using Europa Scientific IRMS with an ANCA-SL preparation module for solid and liquid samples (PDZ Europa Ltd, Grewe, UK) as previously reported (Ogrinc et al. 2012). Each sample was acidified using 1 N HCl to remove carbonate minerals and dried. Stable isotope results are expressed as δ13C or δ15N values in per mil (‰) relative to the VPDB and AIR international standard, respectively. The precision of measurements was ±0.2‰ for δ13C and ±0.3‰ (‰) ± 0.3 (‰) (‰) for δ15N.

The powdered material (2g sample) is then extracted by ultrasonication with an organic solvent (e.g., chloroform/methanol, 2:1 v/v) and evaporated to dryness under a gentle stream of nitrogen to obtain the total lipid extract (TLE). One portion of extract was trimethylsilylated directly and analysed by high-temperature gas chromatography (HT GC) and where necessary combined gas chromatography/mass spectrometry (GC-MS) analyses were performed to identify the structure of components (Evershed et al. 1990). Further aliquots of the TLE were methylated using BF3/methanol to obtain fatty acid methyl esters (FAMEs) (14%, w/v; 100µl; Sigma Aldrich, Gillingham, UK; at 70°C for 1h). The methyl ester derivatives were extracted with hexane and the solvent removed under nitrogen. FAMEs were re-dissolved in hexane for analysis by GC and GC-combustion-isotope ratio MS (GC-CIRMS) using standard protocols (Evershed et al. 1994; Moltrmann et al. 1999; Greg, Slater 2010; Ogrinc et al. 2012). GC-CIRMS analyses were performed using an Isoprime GV system (Microtech, Manchester, UK). Modern samples were extracted by the same procedure. For GC-CIRMS, the precision on repeated measurements was 0.3‰.

The third TLE aliquot was used to identify the di- and triacylglycerol (DAG, TAG) content, following the procedure described by Sigrid Mirabaud et al. (2007). TAG analyses were performed by hybrid quadruple time of flight mass spectrometer (Q-TOF Premier) provided with an orthogonal Z-spray ESI interface (ESI-MS, Waters Micromass, Manchester, UK) (Ogrinc et al. 2012).

**The bulk C and N isotope composition**

The determination of the isotopic composition of carbon (C) and nitrogen (N) was possible in 19 samples. The average and standard deviation from potsherd samples are –26.1±1.1‰ and +6.8±2.9‰ for δ13C and δ15N, respectively (Tab. 2). These data fall in the range expected for degraded animal and plant tissues whose subsistence was based mainly on C3 plants. The δ15N values show greater variations compared to the δ13C values.

We could discriminate three groups of samples on the basis of their δ15N and δ13C values (i.e. δ13C18:0 – δ13C16:0). The first group has the highest Δ13C values of >2.0‰ and were found in two samples (MaTr470 and MaTr599). Freshwater fish is the most likely component of residuals with the highest δ15N value of +10.3‰ in sample MaTr470, while the lower value of +8.4‰ could indicate the presence of molluscs and crustaceans in sample MaTr599. The second group with δ15N values between +0.1 and +5.4‰ and Δ13C values of around 0‰ shows that these pots were probably used to process herbivore products and/or plant material (MaTr53, MaTr100, MaTr105, MaTr118, MaTr161).

**Sample** | **δ13C16:0 (%)**<br> 108 | **δ13C18:0 (%)**<br> 108 | **Δ13C (%)**<br> 108 | **Location / citation**
---|---|---|---|---
Sheep milk | –30.2 | –36.2 | –6.0 | Divača Karst, local
Goat milk | –27.2 | –32.6 | –5.4 | Divača Karst, local
Cow milk | –29.6 | –37.5 | –7.9 | Divača Karst, local
Sheep curd | –27.2 | –32.1 | –4.9 | Divača Karst, local
Sheep cheese | –27.6 | –32.3 | –4.7 | Divača Karst, local
Sheep milk | –33.2 | –39.0 | –5.8 | Spangenberg et al. 2006.7–8, Fig. 4
Sheep milk | –33.2 | –39.9 | –6.7 | Spangenberg et al. 2006.7–8, Fig. 4
Sheep milk | –33.0 | –40.0 | –7.0 | Spangenberg et al. 2006.7–8, Fig. 4
Sheep milk | –33.9 | –41.0 | –7.1 | Spangenberg et al. 2006.7–8, Fig. 4
Cow milk | –29.6 | –34.6 | –5.0 | Richter et al. 2012a,91, Tab. 3
Goat milk | –27.2 | –34.0 | –6.8 | Spangenberg et al. 2006.7–8, Fig. 4
Goat milk | –28.0 | –35.0 | –7.0 | Spangenberg et al. 2006.7–8, Fig. 4
Sheep cheese | –30.8 | –35.8 | –5.0 | Spangenberg et al. 2006.7–8, Fig. 4
Sheep cheese | –29.8 | –35.5 | –5.7 | Spangenberg et al. 2006.7–8, Fig. 4
Goat cheese | –26.5 | –30.5 | –4.0 | Spangenberg et al. 2006.7–8, Fig. 4

**Tab. 3.** Means for stable carbon isotope composition of C16:0 and C18:0 in different dietary components of modern reference animal fats.
MaTr151, MaTr606). The δ13C values of the third group of samples indicate the presence of ruminant adipose and dairy fats (MaTr107, MaTr112, MaTr115, MaTr116, MaTr130, MaTr137, MatR145, MaTr147, MaTr159, MaTr173, MaTr174, MaTr600, MaTr602, 79MT, 87MT, 159MT, 161MT); and the δ15N values ranged from +5.4 to +9.7‰. It should be noted that the δ15N values of protein from terrestrial herbivores from temperate environments in Europe should not exceed 7.0‰ (Richards et al. 2003). However, the protein derived from domestic animals can be higher (Privat et al. 2002; Polet, Katzenberg 2003; Richards et al. 2003; Ogrinc, Budja 2005). As most of the δ15N values of our samples are higher than +7.0‰, we hypothesise that the protein derived mainly from domestic animals.

The evidence of processing of dairy and other animal and/or plant products in pottery vessels was further investigated by lipid analysis. Lipid preservation was very good, with more than 80% of potsherds yielding an appreciable lipid concentration (Tab. 2).

**Processing of dairy and other animal products**

**DAG and TAGs** are indicative lipids of degraded animal fats and could help to differentiate between fats of ruminant and non-ruminant animals and ruminant dairy fats (Kimpe et al. 2002; Mirabaud et al. 2007; Regert 2011). The TAG analysis of modern fats exhibits two main distributions of TAGs: a narrow one that corresponds to non-ruminant adipose fats, and a broad one ranging from T40/T42 to T54, that are characteristic of ruminant or dairy fats (Dudd et al. 1999; Mukherjee et al. 2007). The distribution with low quantities of T42, T44, and T46 are attributed to ruminant adipose fats. It should be mentioned, however, that TAGs are likely to be preferentially degraded. Degradation pathways are not fully understood and might differ depending on the use and burial environment. Therefore, TAG distribution has to be considered as preliminary information to assess the origin of fats.

The identification of TAGs in our samples was first performed with HT GC-MS. MAGs, DAGs and TAGs were detected in 12 samples, together with relatively high amounts of C16:0 and C18:0 fatty acids. A relatively broad TAG distribution in the range from T44 to T54, maximising at T50 or T54, was observed in five samples (159MT, 79MT, MaTr130, MaTr174, MaTr602) indicating the presence of ruminant adipose or dairy fats. Two samples (MaTr115, MaTr174) presented a narrower distribution, from T50 to T54, indicating the presence of ruminant adipose fats.

The identification of original products was further examined by ESI Q-TOF MS and ESI-MS/MS methods to obtain more information about the structure of TAGs. This method enables the TAGs identification down to T28 and could be used to detect dairy fats (Garnier et al. 2007; Mirabaud et al. 2007; Regert 2011) since the HT GC could not detect TAGs under T40. The distribution of TAGs obtained by ESI Q-TOF MS was possible only for three samples (MaTr115, MaTr130, 79MT). The TAGs distribution in MaTr115 was closer to that observed by HT GC: T42 - T52 instead of T50 - T54 (T54 was not detected by ESI Q-TOF MS probably due to its poor ionisation yield).

In the other two samples, 79MT and MaTr130, a large TAG distribution from T28 to T52 was observed,
which indicates the presence of dairy fat products (Fig. 6). The comparison of these TAG and DAG distributions with data on goat versus cow milk favours goat milk (Mirabaud et al. 2007). A more precise differentiation between cow and goat milk could be obtained from fatty acid distribution in T_{4:0} using ESI-MS/MS fragmentation, but this was not possible due to the low quantity and poor ionisation yield of T_{4:0} in our sample; thus a more precise discrimination of the specific origin of dairy fat could not be performed.

The stable carbon isotope composition of individual fatty acids is a complementary tool in determining the origin of residues in ancient pottery vessels. In Figure 7, we present the δ^{13}C values of modern reference animal fats of both Neolithic domesticates and the animals that are actually bred in the region, as well as the values from the Mala Triglavca pottery samples. The δ^{13}C_{16:0} and δ^{13}C_{18:0} values in different reference animal fats ranged from −37.5 to −27.2‰ (Tab. 2). The modern reference animals (cow, sheep and goat) were fed exclusively on C3 local forage grasses. The theoretical mixing curves were determined from the modern reference animal fats, as in Simon E. Woodbury et al. (1995), to illustrate the δ^{13}C values which would result from mixing sheep and porcine fats in the vessels. Each dot in Figure 7 indicates the effect of mixing a specific percentage of each of the respective commodities and their influence on the δ^{13}C_{16:0} and δ^{13}C_{18:0} values. It should be further mentioned that modern dairy ruminant fats (milk, butter and cheese; see Tab. 3) have higher δ^{13}C_{16:0} and δ^{13}C_{18:0} values compared to adipose fats, which can be explained by the distinct metabolic pathway of milk fatty acids. The C_{16:0} fatty acid is the major fatty acid produced from fermenting dietary sugars, while the C_{18:0} fatty acid derives mainly from dietary plant fatty acids. These different sources explain why the values are up to 7.9‰ lower δ^{13}C_{18:0} compared to δ^{13}C_{16:0}. In addition, the δ^{13}C_{16:0} and δ^{13}C_{18:0} values in sheep cheese and curd are enriched in δ^{13}C relative to the raw milk samples. This enrichment is probably the consequence of the bacterial degradation of long-chain fatty acids during cheese or curd production and storage. These data are in good agreement with the published data on reference modern animal fats (Spangenberg et al. 2006; Richter et al. 2012a; 2012b).

Since the data points plot between the reference animal fat fields presented in Figure 7, extensive mixing of animal products could be identified. The δ^{13}C_{16:0} and δ^{13}C_{18:0} values from four samples (MaTr130, MaTr137, MaTr602, 161MT) plot within the area of the mixture between adipose and dairy fats, while another seven samples (MaTr145, MaTr147, MaTr159, MaTr173, MaTr600, 79MT, 87MT) plot within the area of non-ruminant adipose fat. Further four samples (MaTr100, MaTr112, MaTr151, 159MT) plot in the area between porcine and ruminant adipose fat, and the remaining seven samples (MaTr53, MaTr107, MaTr115, MaTr116, MaTr470, MaTr599, MaTr606) do not plot along any of the theoretical mixing curves, thus suggesting an admixture of fats of different origins and different degrees of degradation. Only one sherd plots in the area of dairy fat (MaTr174).

A more precise differentiation between non-ruminant adipose, ruminant adipose and ruminant dairy fats can be obtained in the diagram, where Δ^{13}C values (δ^{13}C_{18:0}−δ^{13}C_{16:0}) are plotted against the δ^{13}C_{16:0} values (Fig. 8). Δ^{13}C values of lower than −3.5‰ are used as a criterion for determining dairy foods (Evershed et al. 2002; 2008; Copley et al. 2003; 2005; Mukherjee et al. 2007; Dunne et al. 2012). Four vessels...
sels from Mala Triglavca (Ma-Tr174, MaTr137, 161MT, 87-MT) are plotted in this region, while another three (MaTr-130, MaTr602, 79MT) are plotted on the border of two ranges, thus suggesting mixing of different types of fats during pottery use. In total, 30% of the pottery samples contained lipids characteristic of dairy fats, indicating that the processing of dairy products in vessels was quite extensive during the Neolithic period at Mala Triglavca.

As presented in Figure 8, 43% of pottery samples plot in the range for ruminant adipose fats. The $C_{16:0}/C_{18:0}$ ratios for these samples range between 1.3 and 2.0 (Tab. 2), which is typical for ruminant adipose fat (Copley et al. 2005). The $\delta^{15}N$ values suggest that people at Mala Triglavca used diverse domesticated animal products (from cattle, sheep and goats) in their diet. A further 17% of the samples (MaTr53, MaTr145, MaTr606, 159MT) fall close to the limit value between non-ruminant and ruminant meat ($\Delta^{13}C = 0$‰). However, the later samples may not be assigned exclusively to meat mixtures, but also to a mixture of plant and animal fats. Two of the samples (MaTr470, MaTr599) plot in the area of non-ruminant fats, and their high $\delta^{15}N$ values of +8.4 and 10.3‰ (Tab. 2) suggest the presence of fresh-water organisms such as molluscs and fish fats. No evidence of porcine fat was found in the pottery when observing the $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids.

**Fig. 8. Plot showing the difference in the $\delta^{13}C$ values of $C_{18:0}$ and $C_{16:0}$ fatty acids ($\Delta^{13}C$) versus $\delta^{15}N$ recovered from pottery extracts from Mala Triglavca and modern reference fats (see Tabs. 2 and 3). The $\star$ represent those with typical degraded dairy TAG distribution.**

The presence of wax esters, characteristic of beeswax, may indicate the addition of honey to other food or the application of beeswax to pottery vessels to improve their impermeability (Regert et al. 2001; Evershed et al. 2003; Copley et al. 2005). Six pottery samples contained beeswax-derived lipids; two of them also yielded evidence of fresh-water fish (MaTr470, MaTr599) and a further four samples contained animal fats and beeswax (MaTr107, MaTr145, MaTr53, MaTr159). Although the quantity is relatively low (21% of all of the samples), it indicates that this particular commodity was utilised in the Mala Triglavca vessels associated with cooking/processing food, or applied as a coating, which made them waterproof and more resistant.

**Different use of vessels**

The results obtained from lipid analyses indicate markedly different uses of the Mala Triglavca pottery. It was found that 30% of sampled pottery con-
tain lipids characteristic of dairy fats (MaTr174, MaTr137, 161MT, 87MT, MaTr130, MaTr602, 79MT), indicating that the processing of dairy products in pottery vessels was quite extensive. The TAG distributions in samples MaTr130 and 79MT showed the residues of dairy products that probably derived from goat milk. We have found evidence that five vessels were used to process only animal fats either of ruminant origin (MaTr116, MaTr173, MaTr600) or a mixture of ruminant and non-ruminant origin (MaTr53, MaTr100). However, none of the total lipid extracts contained porcine adipose fat. The appearance of both animal and plant biomarkers observed in 14 pottery samples (see Tab. 2) suggests mixed food processing and storage. Only one of them (MaTr606) was mainly used in preparing or storing plant foods. Moreover, our results show that some vessels were also used to process aquatic organisms such as molluscs and fish (MaTr470, MaTr599). The presence of beeswax in some of the vessels suggests the storage and use of honey or, more probably, the use of beeswax for waterproofing.

The lipid analysis of Neolithic vessels from Mala Triglavca showed a variety of different foods prepared and consumed at the site (Tab. 2). If we look closely at the most common vessels type at the site, i.e. the Vlaška bowl, we can observe that lipids of ruminant adipose and dairy fats are the most common residue, but freshwater fish, plants and a mixture of different fats were also detected in the bowls. Therefore, we may conclude that these bowls had no specific function, but were rather treated as universal vessels for preparing and consuming a variety of different foods. Typical Vlaška bowls also contained lipids derived from beeswax, which can indicate the technique of applying beeswax to waterproof vessels, but can also suggest the use of honey in food preparation. Other types of vessel showed the use of ruminant adipose and dairy fats in dishes (MaTr107, MaTr174) and ruminant fats in one pot from Mala Triglavca (MaTr173).

The remains of dairy lipids were detected in 29% of the samples, most of them Vlaška bowls (MaTr130, MaTr137, MaTr602, 79MT, 161MT), but also in a beaker (87MT; Mlekuž et al. 2008.247) and a dish (MaTr174), well embedded in the time span 5467–5227 calBC. Sample MaTr130 with an incised plant motif and sample 161MT with incised pendant triangles (Fig. 5.3–4) (Mlekuž et al. 2008. Fig. 6; Šoberl et al. 2008.Tab. 1) are two of the most prominent Vlaška vessels with preserved dairy fats from the site.

Conclusions

The Mala Triglavca case study shows that the Early Neolithic economy in Caput Adriae was mixed. It consisted of both milk and processed milk (low-lactose food), meat (ruminants and non-ruminants) animal products, and fresh-water fish and various plants. The Vlaška group herders managed a broader spectrum of resources than ovicaprids alone, and by fermenting milk they were able to produce a wide range of low-lactose, storable products.

Archaeological and biochemical data suggest that dairying was adopted in the Early Neolithic in Europe. Archaeogenetic data show, on the contrary, the absence of the allelic variant -13 910*T in Neolithic populations in Europe, and that their lactase persistence was very low and may have even been zero. Thus pastoralism appeared before lactase persistence arose or became frequent. We may assume, therefore, that under normal circumstances lactase persistence is not necessarily under very strong selection in these populations and fits with the hypothesis that dairying, milk consumption and fermented milk consumption emerged before the genetic adaptation.

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