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New insights into the subsistence economy of the Eneolithic Dereivka culture of the Ukrainian North-Pontic region through lipid residues analysis of pottery vessels

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Abstract

The Dereivka site of the North-Pontic forest-steppe has been widely investigated because of its potential as a centre for horse domestication (Levine, 1990; Telegin, 1986). Despite the significant archaeological evidence available, Dereivka is considered a contradictory and complex site (Rassamakin, 1999: 143) due to a range of challenges connected with reconciling the various lines of available archaeological evidence. Consequently, a generally acceptable subsistence economic model has still to be developed, with contrasting theories remaining unresolved. This paper presents new results of organic residues analyses from the site. Forty potsherds were submitted to biomolecular and stable carbon and hydrogen isotope analyses and the results discussed in relation to previously published zooarchaeological evidence (Bibikova, 1986; Levine, 1999; Kaiser, 2010). The findings offer a further perspective on the overall subsistence economic strategies of the community, particularly in relation to the exploitation of the horse. Significantly, the biomolecular and stable carbon isotope results confirmed that Dereivka community consumed horse products predominantly, together with smaller proportions of ruminant and non-ruminant products. Interestingly, although ruminant adipose fats were recovered from some vessels, evidence of ruminant dairy product exploitation was insignificant, with only one residue displaying a possible ruminant dairy fat origin. Hydrogen isotope analysis of lipids was applied to investigate equine milk processing in pots (Outram et al., 2009) but these analyses did not offer significant new insights.

1. Introduction

Dereivka was discovered in 1959 (Telegin, 1986), and extensively excavated between 1960 and 1967. The site, located on a promontory of the River Omelnik, a tributary of the Dnieper River (Figure 1), included a settlement from which ceramic sherds were recovered, together with two cemeteries. The finds recovered in the settlement points to Dereivka being influenced by the Tripolye culture (Rassamakin, 1999: 143-149; Telegin, 1986: 36). Although the chronology is still to be resolved, Dereivka has been attributed to the Middle Eneolithic period (3800/3700-3500/3400 BC; Rassamakin, 1999: 127-129), with seven new radiocarbon dates supporting this chronological attribution, placing Dereivka between 3700 and 3530 BC or 3950 and 3530 BC (Rassamakin & Kaiser, in press.).

Figure 1. Location of Dereivka site along the Dnieper River, in the North-Pontic forest-steppe. The location of Botai site in Kazakhstan is also indicated, as it will be mentioned in later discussions.

Rassamakin (1999) describes Dereivka as a permanent settlement with a subsistence economy based on: (i) primitive hoe agriculture, as cereal imprints are seen in ceramic vessels (Pashkevych, 2012; the possibility of using cereal imprints as an indication for agriculture is discussed by Motuzaite-Matuzeviciute, 2012, 2014); several agricultural tools (Telegin, 1986) have also been recovered, and (ii) hunting-fishing activities, indicated by the faunal evidence that displays a predominance of horses, wild animals and fish bones (Table 1; Bibikova, 1986; Rassamakin, 1999) and by isotope evidence obtained from human and animal bones recovered

from the Neolithic cemetery of Dereivka (Lillie et al. 2011) that suggests a diet mainly based on C_3 terrestrial foodstuffs, supplemented with aquatic resources, such as freshwater fish.

Table 1 summarises the different classes of animals inferred from the faunal records from the Dereivka excavations (Bibikova, 1986) and the relative percentages of the main animal groups. It is notable that the number of identified specimen (NISP) and the minimum number of individuals (MNI), the only evidence available at the site, are inconsistent, with the abundance of equines being 59.7% based on NISP, whereas based on the MNI is only 24.2% of the total faunal assemblage. This, together with the absence of additional evidence, has led to contrasting interpretations, notably Anthony & Brown (2003: 58) discarded the possibility that Dereivka people were hunters, believing instead that it was a community of animal breeders (including horses) and arable agriculturalists. Further interpretations are not helped by the fact that animal exploitation by the Tripolye culture, believed to be the major influence on Dereivka community, has not been extensively investigated (Rassamakin, 1999) so does not offer a general comparative model.

Besides the existing evidence, an accepted reconstruction of the Dereivka subsistence economic strategy, a common theory about the extent of the animal exploitation and specifically the importance of horse domestication has yet to be achieved and remains a subject of major interest in Eurasian prehistoric studies.

Table 1
The different classes of animals inferred from faunal records in Dereivka site (Bibikova, 1986). Both MNI and NISP are shown. Sheep and goats are combined; the wild animals includes red deer, roe deer, boar, elk, badger, beaver, wolf, fox, hare, bear and otter.

	Cattle	*Sheep/Goats*	*Pigs*	*Horses*	*Wild animals*	*Fishes*
MNI	18	16	9	52	83	37
MNI%	8.4	7.4	4.2	24.2	38.6	17.2
NISP	618	88	114	2412	673	136
NISP%	15.3	2.2	2.8	59.7	16.7	3.4

1.1 Horse domestication

Several questions exist surrounding the matter of horse domestication (Levine, 1999) including: (i) the reasons why horses were first domesticated, i.e. for either meat, riding and/or traction; (ii) the period when this important knowledge was firstly attained, i.e. Neolithic, Eneolithic or Bronze Age; and (iii) the location of the first centre(s) of horse domestication, considered by the

majority to be the Eurasian steppe (Levine, 1999; Olsen, 2006; Sherratt, 2003; Stear, 2008; Vilà, 2001; Warmuth, 2012; Outram et al., 2009). This paper will not answer to all these questions but it will attempt to add new evidence to an archaeological question that for decades has intrigued a wide range of scholars (e.g. Anthony, 2010; Bibikova, 1969; Bunyatyan, 2003; Dietz, 2003; Levine, 2005; Sherratt, 2003; Telegin, 1986). After all, the domestication of horse was a major event that changed the way of life both socially and economically, revolutionizing transportation, communication and trade. Critically, wild horses were a common part of the fauna in the Eurasian steppe (Levine, 2006: 193), and prior to domestication, these animals were frequently hunted in the Eastern steppe (Benecke & Von den Driesch, 2003). Later, the horse became mainly important for secondary products and as a possible transport instrument (Olsen, 2006) for use in the herding of other animals or even a weapon during conflicts (Anthony, 2010). However, the domestication of horse represents a particularly controversial matter in zooarchaeology as: (i) there are no generally accepted osteological differences between wild and domesticated horses (Levine, 2005: 11) especially in the initial domestication stages (Bökönyi, 1974; Clutton-Brock, 1999; Olsen, 2006), and (ii) mortality profiles are particularly challenging to interpret (Olsen, 2006: 248).

Dereivka has been central to discussions of horse domestication since 1967 (e.g. Anthony & Brown, 1991; 2003; Bibikova, 1969; Bökönyi, 1974; Levine 1990; Rassamakin, 1999; Telegin, 1986). The evidence of horse domestication from Dereivka is indirect, primarily inferred from bone and artefactual evidence (Levine, 2005), however, interpretations are compromised by the fact that a large number of bones were lost due to ineffective curation post-excavation. Hence, the main indirect evidence of horse exploitation at Dereivka is the recorded high relative abundances of equine bones (Levine, 2006: 193; Telegin, 1986: 84), however, the high proportion of equine bones might be interpreted as arising through increasing horse hunting rather than its domestication (Levine, 2006: 193). After Bibikova (1986) Levine carried out zooarchaeological metrical analyses of 900 bones and teeth in Dereivka (Levine, 1990; 2005) during which she was able to distinguish between equine bones of adult males and females discovering that the ratio between males and females was 9:1. The latter was interpreted as evidence of a selective hunting technique or 'stalking model', in which the prey is approached by stealth and killed (Levine, 1990: 736). In addition, Levine pointed out that the majority of the equine teeth from Dereivka belonged to individuals between 5 and 8 years old, which are the most productive years of a horse (Levine, 1990: 738). It was argued that if the horses from

Dereivka were domesticated for meat they would have been killed at 2 or 3 years old, when maximum size and hence maximum meat yield was reached. In contrast, if they were domesticated for secondary products, they would have been slaughtered after the age of 15 or 16 years old in order to exploit the secondary products as long as possible (Levine, 1990: 738). Therefore, the latter analysis points to the Dereivka horses being a predominantly wild assemblage.

Three conditions are considered to be required for the domestication of a species (Kuzmina, 2003): (i) the presence of a wild ancestor, (ii) the requirement for food that could only be met through domestication, and (iii) the possession of sophisticated herding skills (Kuzmina, 2003: 209). The first two conditions have been broadly discussed in the past (Rassamakin, 1999: 134) and appear to have existed in the North-Pontic region (Kuzmina, 2003: 209). Concerning the third prerequisite, it is generally believed that domesticated ruminants appeared in the North-Pontic region at around the 6th millennium BC (Bunyatyan, 2003; Kotova, 2003; Kuzmina, 2003) and, given the 20% (NISP) of domesticated animal bones in the faunal record, it can be assumed that the Eneolithic Dereivka people were at an initial phase of animal domestication. Nevertheless, others strongly believe in a later introduction of domesticated animals in the North-Pontic region (e.g. Wechler, 2001), or at least in some areas, depending on the local-regional ecosystem (Rassamakin, 1999). Thus, providing direct evidence for the Dereivka community possessing advanced 'herding skills', such as dairying based on milk fat residues in pottery, would lend concrete support to this hypothesis.

In an effort to test the latter hypothesis, herein, we explore a new source of information regarding the animal exploitation and management at Dereivka, namely animal fat residues preserved in cooking pots. This approach has been widely used to investigate the processing of animal product in pottery at both the new and old world sites (e.g. Craig et al., 2005; Evershed et al., 2008a, b; Spangenberg et al., 2006; Lantos et al., 2015). The identification of fats is achieved through gas chromatographic and mass spectrometric analyses, with further classification, i.e. ruminant *versus* non-ruminant, ruminant carcass *versus* diary, terrestrial *versus* aquatic, attained through compound-specific stable isotope analysis (Evershed et al, 1997; 2002; Hansel & Evershed, 2009; Cramp & Evershed, 2014; Cramp et al., 2014). Of particular relevance to this investigation is the study of Outram et al. (2009) who combined zooarchaeological information with organic residue data from pottery to provide new evidence for horse domestication at the

Eneolithic site of Botai in Kazakhstan (Figure 1). The new zooarchaeological approaches cannot now be applied at Dereivka due to loss of the faunal collection. However, we can apply the organic residue approach, including a novel dual isotope carbon and hydrogen isotope-based protocol to look for evidence for horse milk and carcass product processing in pottery. Hence, lipid residue analyses have the potential to provide evidence for the management strategies used by the Dereivka people for ruminants *versus* horses (*cf.* Outram et al., 2012), and possibly the exploitation of aquatic resources (*cf.* Cramp et al. 2014).

2. Material and methods

2.1 Glassware, solvents and reagents

All solvents used were HPLC grade (Rathburn) and the reagents were analytical grade (>98% purity). Reusable glassware was washed with Deacon 90 (Deacon Laboratories), rinsed with acetone, oven dried and, when possible, furnaced at 450°C for 4 h. Analytical blanks were prepared with each batch of samples to monitor for possible sources of contamination in solvents and reagents.

2.2 Archaeological pottery

A total of 40 potsherds from the Eneolithic Dereivka site were subjected to organic residue analysis. The sample selection was carried out at the archaeological Institute of Kiev and supervised by the archaeologist Yuri Rassamakin. Sherds likely to have been used in cooking processes were chosen, the great majority of the sherds were previously cleaned by archaeologists, and therefore no presence of burning area that could have indicated use in cooking were seen. However, rim and body of the sherds were mainly selected as these have been proven to contain higher concentrations of solvent extractable lipid (Charters et al., 1993).

2.3 Modern reference animal fats

The identification of the archaeological fats has been realized by comparing the archaeological stable isotope composition with those of a broad reference database of modern fats. The database comprises compound-specific $\delta^{13}C$ values of the palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) fatty acids of modern reference dairy fats, ruminant adipose fats and non-ruminant adipose fats from Europe (Copley et al. 2003; Salque et al., 2012; Spangenberg et al., 2006), Asia (Outram et al., 2009; Pitter, et al., 2012) and Africa (Dunne et al., 2012) In addition modern Kazakh equine and freshwater fish fats have been added to the database (Chivall, 2008; Outram et al., 2009; Stear, 2008).

186

187 2.4 Solvent extraction of lipid residues from archaeological pottery

188 About 2 g of potsherd surface was cleaned using a modelling drill to remove exogenous residues.
189 The cleaned sample was grounded in a glass pestle and mortar; the fine powder was weighed and
190 placed in a glass vial. An internal standard (20 μg of n-tetratriacontane) was added to the
191 powdered sherd to enable the quantification of lipid extract. Lipids were extracted using $CHCl_3$-
192 MeOH (10 ml; 2:1 v/v) by ultra-sonication (2 x 20 min). After centrifugation in a test tube
193 (2,500 rpm, 10 min), the total lipid extract (TLE) was evaporated under a gentle stream of
194 nitrogen to 3 mL. Aliquots were derivatised with BSTFA (70°C, 1 h) for high temperature GC
195 analysis (Evershed et al., 1990; Charters et al., 1993a). Further TLE aliquots were hydrolysed
196 with 0.5 M NaOH in MeOH-H_2O solution (5 ml; 9:1 v/v, 70°C). The neutral fraction was
197 extracted with hexane (3 x 3 mL) in a clean glass vial and store in the refrigerator until required
198 for analysis. Finally, the methanol fraction was acidified to pH 3 with 1 M HCl and the fatty
199 acids extracted with chloroform (3 x 3 mL) for the archaeological fats. The extracted fatty acids
200 in solvent were evaporated under gentle stream of nitrogen and treated with 100uL of
201 BF_3/MeOH (Sigma Aldrich; 14% w/v, 70°C, 1 h). After allowing to cool, dichloromethane
202 (DCM) extracted double-distilled water was added (1 mL) and FAMEs extracted (3 x 2 mL)
203 with chloroform. The solvent was evaporated to dryness under a gentle stream of nitrogen and
204 the FAMEs stored in freezer until required for analysis. The FAMEs were dissolved in hexane
205 for analyses by GC, GC/MS and GC/C/IRMS.

206

207 2.5 Direct methanolic acid extraction

208 Lipids were extracted from the powdered sherd in a culture tube (I) by adding 5 mL of a H_2SO_4–
209 MeOH and heating (2% *v/v*, 70°C, 1 h, vortex mixing every 5 min). Then the H_2SO_4–MeOH
210 solution containing the extract was transferred to a test tube and centrifuged (2500 r.p.m., 10
211 min). The clear supernatant was transferred to clean culture tube (II) and 2 mL of (DCM)
212 extracted double-distilled water added. For a total organic residues extraction, 3 mL of hexane
213 were added to the extracted potsherd in the culture tube (I) to recover any lipids not fully
214 solubilised by the methanol solution. The hexane supernatant was then transferred to the H_2SO_4–
215 MeOH solution in culture tube (II) and vortex mixed to extract the lipids - the washing of the
216 sherd with hexane and vortex mixed in culture tube (II) was repeated twice with the hexane
217 being transferred to a clean vial for blowing down. Following this, 2 mL hexane were added
218 directly to the H_2SO_4–MeOH solution in culture tube (II) and vortex mixed to extract remaining

lipid residues (x2). The hexane extracts were combined and evaporated to dryness under a gentle nitrogen stream and re-dissolved in 1 mL of hexane to give the hydrolysed/transmethylated total lipid extract (TLE). In case *n*-alkanols are present in the lipid extract, an aliquot is treated with BSTFA (70°C, 1 h) for GC, GC/MS and GC/IRMS analyses.

2.6 Instrumental analysis

2.6.1 High Temperature-Gas Chromatography (HT/GC)

HT/GC analyses were performed on an Agilent Technologies 7890A GC System. Trimethylsilylated (TMS) total lipid extracts (1 µL) were injected through an on-column injector, in track-oven mode onto a 15 m x 0.32 mm i.d. fused silica capillary column coated with a dimethyl polysiloxane stationary phase (non-polar column, 100% DB1-HT, 0.1 µm film thickness; Agilent Technologies). The carrier gas was helium and the GC oven temperature program was 50°C (2 min) to 350°C (10 min) at a rate of 10°C min^{-1}, followed by an isothermal hold at 50°C for 1 min (Charters et al., 1993a; Evershed et al., 1990). A flame ionisation detector (FID) was used to monitor the column effluent. Peaks were identified by comparison of retention times with those of derivatised external standards. Finally, quantification was achieved by the internal standard method.

2.6.2 Gas Chromatography-Mass spectrometry (GC/MS)

The GC/MS analyses for the detection of the TMS were performed on a ThermoFinnigan Trace MS operating with an ionizing energy (IE) of 70eV with a GC interface temperature was 300°C and a source temperature of 200°C. The scanning range was *m/z* 50-650. Samples were introduced by on-column injection. The analytical column was a 60 m x 0.32 mm coated with polymethylsiloxane (non-polar column, ZB-1, 0.12 µm film thickness, Phenomenex). The carrier gas was helium and the temperature program was 50°C (2 min) to 300°C (10 min) at a rate of 10°C.min^{-1}, followed by an isothermal hold at 50°C (1 min). Data acquisition and processing were carried out using XCalibur software. GC/MS analyses of FAME derivatives were performed using a Finnigan Trace quadrupole MS, operated in electron source (EI) mode operating at 70eV with a GC interface temperature of 250°C and a source of temperature of 200°C. The scanning rate was between *m/z* 50-650. Diluted samples were introduced using a PTV injector onto a 60 m x 0.32 mm i.d. fused silica capillary column coated with cyanopropyl-methylpolysiloxane stationary phase (polar column, 50%, VF-23 ms, 0.15 µm film thickness; Varian, Factor Four). The carrier gas was helium and the temperature program was 50°C (2 min)

to 250°C at the rate of 10°C min^{-1}, followed by an isothermal hold at 50°C (1 min). For the detection of ω-(*o*-alkylphenyl)alkanoic acids (APAAs), the MS was operated in total ion current (TIC) and selected ion monitoring (SIM) mode acquiring at *m/z* 105, 262, 290, 312 (Cramp & Evershed, 2014). Data acquisition and processing were carried out using XCalibur software).

2.6.3 Gas Chromatography/Combustion/Isotope Ratio Mass spectrometry (GC/C/IRMS)

Compound/specific stable Carbon isotope ratios were performed using a GC Agilent Technologies 7890A coupled to an IsoPrime 100 (EI, 70eV, three faraday cup collectors *m/z* 44, 45 and 46) via an Isoprime GC5 combustion interface with a CuO and silver wool reactor maintained at 850°C. Lipid extracts were analysed using a cyanopropyl/methylpolysiloxane stationary phase (Polar column, 50% VF/23ms, 60 m x 0.32 mm i.d., 0.15 mm film thickness). Helium was used as carrier gas and the temperature programme was the same as for the GC/MS analyses.

2.6.4 Gas Chromatography/Thermal Conversion /Isotope Ratio Mass spectrometry (GC/TC/IRMS)

Compound-specific stable hydrogen isotope ratios were performed using a ThermoFisher Scientific Delta^{Plus} V GC/TC/IRMS (TC reactor, 300 x 0.5 mm i.d.; Al_2O_3; 1450°C). FAMEs were introduced to the GC via Agilent PTV injector (splitless mode; 50-300°C; purge time=1 min) and later an Agilent Split/Splitless injector (splitless mode; 300°C purge time=2 min). Lipid extracts were analysed using a fused silica capillary column (30 m × 0.25 mm i.d.) with a methylpolysiloxane stationary phase (Zebron ZB-1; 0.25 μm film thickness). Faraday cups were used for the detection of ions of *m/z* = 2 (H_2^+) and *m/z* = 3 (HD^+) with cup centring performed using the HD^+ ion beam. A retardation lens removed $^4He^+$ ions and in order to correct for H^{3+} ions a calibration was performed every day using Thermo Finnigan ISODAT 2.0 software; the H^{3+} factor was typically below 5 and had a rate of change of less than 0.1 day^{-1}.

3. Results

3.1 Lipid quantification and composition

Lipid preservation was good with a total of 75.5 % of potsherds (n=31) yielding appreciable lipid concentrations (>5 μg g^{-1}); however, due to post-excavation contamination of 4 of the extracts, only 27 extracts were submitted to GC/C/IRMS. Figure 2 displays two typical chromatograms occurring in Dereivka potsherd extracts. The most common distribution was dominated by fatty

acids that generally range from $C_{12:0}$ to $C_{24:0}$ acyl carbon atoms with high abundances of the $C_{16:0}$ and $C_{18:0}$ fatty acids, which are indicative of the presence of degraded animal products (e.g. Evershed et al., 1997). The general abundance of branched-chain fatty acids suggested bacterial origin diagnostic of ruminant animal fat (Christie, 2012). However, branched-chain fatty acids (especially iso- and anteiso-$C_{17:0}$) are also detected in equine adipose fats and likely derive from similar groups of microorganism located in the hindgut of the horse (Hintz & Cymbaluk, 1994; Pond et al., 1995). Finally, a number of residues displaying short chain fatty acids (mainly $C_{14:0}$) that may suggest dairy fats (Christie, 1983; Kuksis et al., 1973) were also detected. However, short-chain saturated fatty acids are detected very rarely in archaeological pottery, due to their compositional alteration during burial to a distribution more resembling adipose fats (Dudd & Evershed, 1998), and thus cannot be used as reliable diagnostic criteria for milk processing.

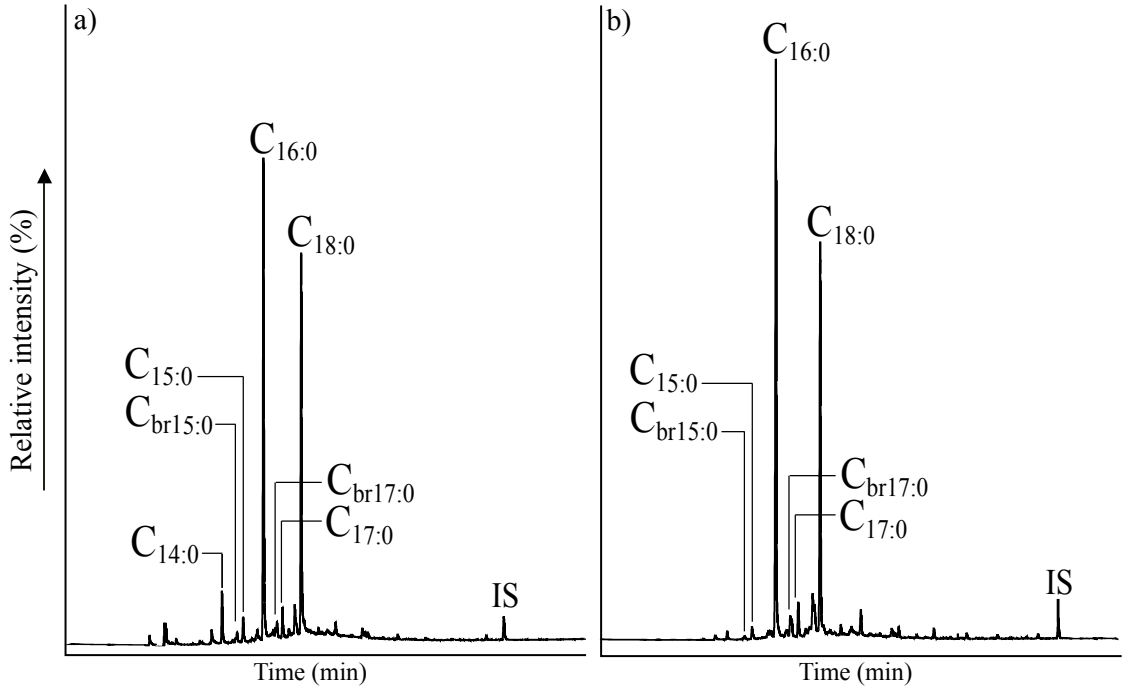

Figure 2. Partial gas chromatogram of TLEs from sherd (a) DER16 and (b) DER25 showing $C_{15:0}$, $C_{16:0}$, $C_{17:0}$ and $C_{18:0}$ fatty acids (F); C_{br15}, C_{br17} are branched-chain F. The internal standard (IS) is C_{34} *n*-tetratriacontane.

3.2 Carbon isotope composition

Figure 3 displays the results of absorbed lipid residue analysis on potsherds from 27 extracts. The $\delta^{13}C$ values of $C_{16:0}$ fatty acid plotted against $C_{18:0}$ fatty acid are shown in Figure 3a. $\delta^{13}C_{16:0}$ range from -32.2‰ to -25.7‰, whereas $\delta^{13}C_{18:0}$ values range between -31.7‰ and -24.9‰ with mean values, respectively, of -29.1‰ and -29.3‰. Figure 3b shows the $\Delta^{13}C$ plot ($\delta^{13}C_{18:0}$-$\delta^{13}C_{16:0}$), which allows separation of animal fats, by removing environmental effects (Copley et

al., 2003; Evershed, 2008). An appreciable number of Dereivka residues (n=13) exhibit $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids characteristic of equine products (displaying mean $\delta^{13}C_{16:0}$ value of -28.9‰, mean $\delta^{13}C_{18:0}$ value of -29.0‰ and mean $\Delta^{13}C$ value of -0.1‰). Five residues plot in the range of ruminant adipose, of which only one residue overlaps between adipose and dairy fats. Examination of the raw data plot in Figure 3a further reveals that only one residue has characteristic isotopic composition of porcine products (Mukherjee et al., 2007) with $\delta^{13}C_{16:0}$ =-26.7‰, $\delta^{13}C_{18:0}$ =-24.9‰ and $\Delta^{13}C$=1.8 ‰, and that five residues have possible freshwater fish origin, showing more depleted isotopic composition (Cramp & Evershed, 2014) with mean $\delta^{13}C_{16:0}$ value of -31.6‰, mean $\delta^{13}C_{18:0}$ value of -30.7‰ and mean $\Delta^{13}C$ of 1.0 ‰. Three extracts have possible mixed origin (white dots). Finally, the distribution of $\delta^{13}C_{16:0}$ values (Figure 3b) of the majority of the extracts range between -32.2‰ to -25.7‰, which is comparable to the $\delta^{13}C_{16:0}$ values for modern ruminant fats from British animals, raised on a strict C_3 diet ($\delta^{13}C_{16:0}$ values ranging from -30.9‰ and -28.6‰ and -32.9‰ and -30.4‰ for the $\delta^{13}C_{18:0}$; Copley et al., 2003). The latter data suggest that the animals producing these fats were consuming C_3 diets.

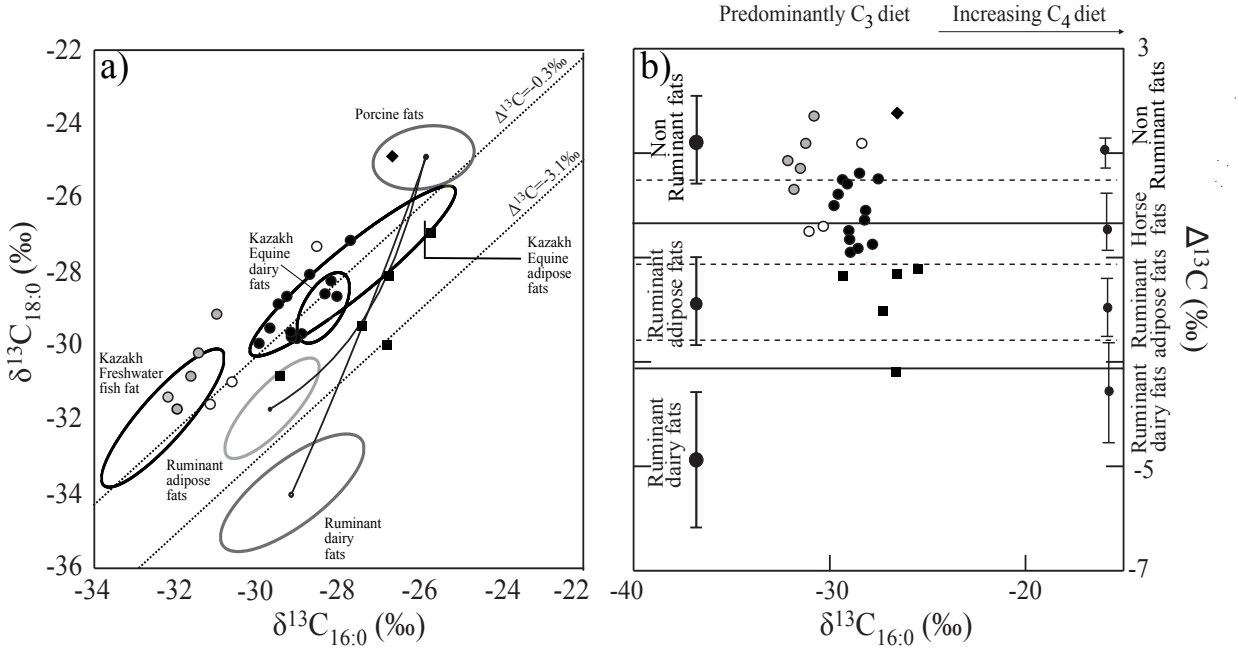

Figure 3. Scatterplots of (a) $\delta^{13}C$ values of $C_{16:0}$ fatty acid against the $C_{18:0}$ fatty acid extracted from 40 pottery vessels from Dereivka. In the plots, equine fats (black dots); porcine fat (black rhombus); freshwater fish fats (grey dots); ruminants fats (black squares); mixed fat residues (white dots). Archaeological values overlay confidence ellipses corresponding to the values obtained from modern reference fats, which enables the species classification of the ancient animal products; and (b) $\delta^{13}C$ values of $C_{16:0}$ against the $\Delta^{13}C$ values ($\delta^{13}C_{18:0}$ - $\delta^{13}C_{16:0}$).

3.3 Hydrogen isotope composition

The research carried out by Outram et al. (2009) was based on the hypothesis that the hydrogen used to biosynthesise equine fats derives ultimately from environmental water, such that carcass fat will represent an integration of hydrogen for the entire period of accumulation, probably many months, while milk fat hydrogen derive from late spring or summer precipitation. These two different fats will exhibit different averaged δD values reflecting the period of biosynthesis (Dansgaard, 1964; Rozanski, 1993). Significantly, Kazakhstan precipitation shows a substantial modern seasonal variation in precipitation δD, of ca. 80‰ in the area of Raïsovka (where Botai was located), with values of -155‰ and -80‰, being recorded in January and July, respectively (Bowen et al., 2009). Interestingly, the seasonal deuterium effect in Kazakhstan appear to have persisted in precipitation over the millennia, allowing the identification of equine dairy residues, enriched by roughly 100‰ (indicated by stars in Figure 4) compared to the main cluster of carcass fat residues. Following this idea the lipid residues from Dereivka attributed to equine fats by compound-specific stable carbon isotope analysis, were submitted to compound-specific stable hydrogen isotope analysis in order to determine if the processing of equine milk products could be detected.

Figure 4 displays the hydrogen isotope results of equine fat residues in potsherds from Dereivka (n=6; indicated by black dots) and Botai (n=42; indicated by white dots and stars; the data for Botai are taken from Outram et al., 2009). Examination of Figure 4 reveals that the 6 Dereivka residues display δD values (mean δD $C_{16:0}$ of -273.6‰ and $C_{18:0}$ of -229.0‰) plotting closer to both the modern reference and Botai horse carcass fats than to horse milk fats.

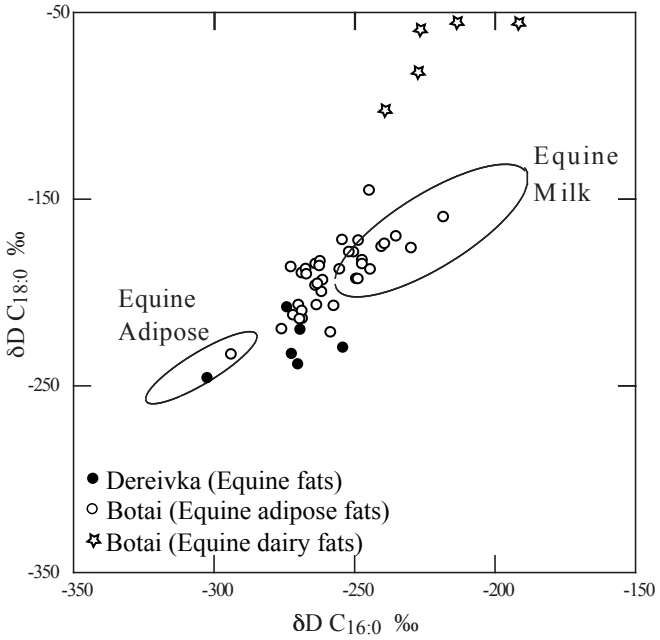

Figure 4. Scatterplot of δD values of $C_{16:0}$ against $C_{18:0}$ fatty acids extracted from (i) six Middle Eneolithic potsherd from Dereivka site (black dots) and (ii) 40 Middle Eneolithic potsherd from Botai site (white dots and stars). The residues were previously attributed to equine products by compound-specific stable carbon isotope analysis. Archaeological values overlay confidence ellipses corresponding to the values obtained from modern Kazakh reference fats.

4. Discussions

The information obtained from existing archaeozoological evidence and from molecular and stable carbon isotope analysis are consistent and revealed that the subsistence economy of Dereivka community was predominantly based on horse exploitation (either wild or domesticated). The organic residue analyses confirm that horses played a significant role in the lives of prehistoric people in the Eneolithic of the North-Pontic forest-steppe (Levine, 1999; 2006). Significantly, only one residue is attributed to non-ruminant, likely porcine products, which is surprising, as the faunal assemblage comprised 5 to 10% pigs and 14% wild animals, including wild boar. It is likely that porcine products are simply not detectable against the background of other animal products processed in the vessels or, much less likely, that they were processed and consumed in an alternative manner, not involving the use of pottery vessels, e.g. using a spit over an open fire. The consumption of freshwater fish is supported by depleted $\delta^{13}C$ values for the $C_{16:0}$ and $C_{18:0}$ fatty acids which reflects the faunal record (Bibikova, 1986) that includes 4 to 15% fish bones. However, no biomarkers for fish (APAA's) were detected, probably suggesting that the cooking temperatures were typically low, as the diagnostic APAA's are produced at temperatures higher than 260-270°C (Evershed, 2008; Hansel & Evershed, 2009). As mentioned in the Introduction, stable isotope analysis of human bones, recovered from

the Neolithic Dereivka cemetery, suggested a diet based on C_3 terrestrial resources supplemented with aquatic resources, such as freshwater fish (Lillie et al. 2011).

Significantly, from the compound-specific stable carbon isotope analyses of the animal fat residues in the pottery, it appears that only 22% of the vessels analysed were used for the processing of ruminant products, which concurs with the *ca*. 20% ruminants represented in the faunal assemblage, which included cattle, sheep and goats (see Table 1). An interesting feature of the organic residue findings is the near absence of pottery vessels showing ruminant dairy products (only one extract out of 27 has a possible ruminant dairy origin, $\Delta^{13}C$ value -3.2‰). The latter suggests that the Dereivka people did not regularly process milk, which is consistent with the low percentage of domestic ruminants observed in the Dereivka faunal records (Bibikova, 1986). This latter finding lends further weight to interpretations that animal domestication was at an initial stage of development, since the exploitation of secondary products, e.g. dairy, is strongly indicative of the existence of a full pastoral economy. The latter is consistent with the theory that Dereivka was a community mainly based on hunting-fishing activities (Rassamakin, 1999). The precise reasons why the Dereivka people had yet to adopt dairying by the 4th millennium BC remains unclear and are likely connected to lactose-intolerance (Salque et al., 2013), environmental constraints and/or cultural belief(s).

While the exploitation of horses is clear from both faunal (Bibikova, 1986; Kaiser, 2010) and pottery lipid record, it is not possible to infer from the animal fat residues in the pottery whether the horses were wild or domesticated due to overlap of the $\delta^{13}C$ values of equine adipose and milk reference fats (Outram et al., 2009; Stear, 2008). For the reasons described above, Botai horse milk and carcass fats were readily separable based on the δD values of their fatty acids. δD values were recorded for a limited number (n=6) of the Dereivka residues, however, the values obtained (Figure 4) point to these residues deriving mainly from equine carcass fats. While this might seem to contrast with the successful detection of horse milking at Botai in Eneolithic Kazakhstan, there are differences between Botai investigation and the present study of Dereivka that are worth emphasising: (i) most importantly a larger difference exists between the δD value of summer and winter precipitation at Botai (80 ‰) than at Dereivka (50 ‰) that, given the precision of the compound-specific δD determinations (±5 ‰), fundamentally limits the capacity to resolve milk and carcass residues, (ii) the sample size studied at Botai was nearly an order of magnitude larger than for Dereivka, which necessarily increased the likelihood of detecting a

low level of horse milking on statistical grounds, and (iii) the interpretation of the deuterium isotope data from Dereivka is complicated by the limited understanding of the factors governing the fractionation of the hydrogen isotopes from the meteoric water to animal tissue (Chivall, 2008; Cormie et al., 1994) exacerbated by the lack of modern reference fats from the region (Mileto, 2016, in prep).

Nevertheless, the lipid residue results provide new insights into the subsistence economic strategies of Dereivka community. As discussed above the absence of ruminant dairy product residues from the Dereivka pottery, suggests a relatively unsophisticated knowledge of ruminant domestication existed in the region, which could imply that the Dereivka horses were wild rather than domesticated (e.g. Kuzmina 2003; Rassamakin 1999; Levine 2005; Anthony 2010). However, this interpretation is countered by the finding of equine dairy fats in Botai pots (Outram et al., 2009), a site essentially devoid of domestic ruminants (Olsen et al., 2006). The implication being that knowledge of horse domestication could have been acquired by communities with no obvious knowledge of ruminant milking (Kuzmina, 2003).

In summary, the lipid analyses of the Dereivka pottery are dominated by degraded animal fats yielding compound-specific stable carbon values of the fatty acids which correspond well with the faunal records. Overall, exploitation of equine products was substantial. However, although the number of organic residues deriving from equine fats was appreciable (48%), it is not possible to infer whether these derived from wild or domesticated horses (Outram et al., 2009). Finally, the lack of ruminant dairy products in Dereivka pots might point to a relatively unsophisticated knowledge of animal domestication which when viewed with zooarchaeological evidence potentially indicates the horses were wild (Levine, 1990). Thus, in the absence of other reliable evidence supporting domestication, we must assume that Dereivka horses were primarily hunted. Resolution of this matter in a way that was achieved at Botai site has been thwarted by the small number of horse fat residues available for deuterium isotope analysis from Dereivka, exacerbated by the differences in the seasonal water cycle between Botai and Dereivka, and the lack of an appropriately curated faunal assemblage for further metrical analysis (Outram et al., 2009).

Conclusions

This research provided an interdisciplinary investigation of diet and subsistence strategies of the human groups that lived in the Middle Eneolithic site of Dereivka, in the forest-steppe of the

North-Pontic region, along the Dnieper River. The combination of existing zooarchaeological evidence and new molecular and stable isotope results provided a number of significant new findings:

(i) The carbon isotope results from the Dereivka pottery organic residues strongly reflect the faunal records, which increases the reliability of both lines of evidence.

(ii) The subsistence economy of Dereivka site was predominantly based on horse exploitation, suggesting that horses played a significant role in the life of this community (Levine, 1999; 2006; Anthony & Brown, 2003; Anthony, 2010);

(iii) Apart from the exploitation of equine products, other animal products were identified in pots, including ruminant adipose products (n=5) and freshwater fish fats (n=5) supporting the findings of Lillie (2011) based on stable isotope analyses of human bones recovered from the Neolithic cemetery of Dereivka. This confirms a diverse subsistence economy of the Dereivka community;

(iv) The compound-specific stable carbon analysis of animal fat residues revealed that ruminant dairy products were not processed in pottery vessels, suggesting that the Dereivka people did not commonly exploit secondary products, supporting the theory that Dereivka people were mainly hunters (Rassamakin, 1999);

(v) The absence of ruminant dairy fats could be considered as further indirect evidence for the absence of horse domestication at Dereivka site as the 'herding skill' is considered a fundamental prerequisite to domesticate wild horses (Kuzmina, 2003);

(vi) Dereivka people extensively used pots to process equine products; however, it was not possible to infer from the carbon of hydrogen isotope data if horses at Dereivka were wild or domesticated;

(vii) It should, however, be emphasised that possibilities remain for the application of the dual carbon of hydrogen isotope approach to investigate horse milking at Dereivka through analyses of a substantially larger pottery assemblage and further assessments of the paleohydrological cycle in the region in order to establish the seasonal range of δD values of precipitation in the region.

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