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## **Regional asynchronicity in dairy production and processing in early farming communities of the northern Mediterranean**

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### **Significance Statement**

This unique research combines the analyses of lipid residues in pottery vessels with slaughter profiles for domesticated ruminants to provide compelling evidence for diverse subsistence strategies in the northern Mediterranean basin during the Neolithic. Our findings show that the exploitation and processing of milk varied across the region, although most communities began to exploit milk as soon as domesticates were introduced between 9-7,000 years ago. This discovery is especially noteworthy as the shift in human subsistence towards milk

production reshaped prehistoric European culture, biology and economy, in ways that are still visible today.

### **Abstract**

In the absence of any direct evidence, the relative importance of meat and dairy productions to Neolithic prehistoric Mediterranean communities has been extensively debated. Here, we combine lipid residue analysis of ceramic vessels with osteo-archaeological age at death analysis from 82 northern Mediterranean and Near Eastern sites dating from the 7<sup>th</sup>-5<sup>th</sup> millennia BC to address this question. The findings show variable intensities in dairy and non-dairy activities in the Mediterranean region with the slaughter profiles of domesticated ruminants mirroring the results of the organic residue analyses. The finding of milk residues in very early Neolithic pottery (7<sup>th</sup> millennium) from both the east and west of the region contrasts with much lower intensities in sites of northern Greece where pig bones are present in higher frequencies compared to other regions. In this region, the slaughter profiles of all domesticated ruminants suggest meat production predominated. Overall, it appears that milk or by-products of milk was an important foodstuff, which may have contributed significantly to the spread of these cultural groups by providing a nourishing and sustainable product for early farming communities.

/body

In southwestern Asia, domestication of sheep, goats and cattle started between 8,500 and 8,000 cal. BC, with morphological traits of domestication being detected in some archaeozoological records from 8,500 cal. BC (1, 2). However, as domesticates started to provide the majority of the meat at Pre-Pottery Neolithic (PPN) sites only one millennium later (3, 4), it has been argued that milk might have been one of the initial attractions of domesticating ruminants (4). The development of archaeozoological methods for reconstructing herd structures allows herd management practices to be inferred from the archaeological faunal record (4-6). Indeed, different types of management strategies of domestic ungulate herds produce different patterns for the slaughtering age of animals (5) assessed through the estimation of dental age at death. Subsistence strategies can thus be understood, providing evidence for the production of meat and milk from ungulates. In parallel to archaeozoology, the characterisation of animal lipids extracted from pottery vessels used in cooking has been demonstrated to be a powerful method for detecting the processing of carcass and dairy products (7, 8). Archaeozoological studies have demonstrated that milk production in the Near East started early in the domestication process in “stock-herding hunter-cultivator” communities (PPN; ref. 3, 4), while dairy residues have been detected in early ceramic containers dating to the 7<sup>th</sup> millennium (9). Current evidence suggest dairying practices developed largely in lactase non-persistent communities, providing the base for the selection of the European Lactase Persistence-associated (LP; 13,910\*T) allele (10); with the allele first appearing in human ancient DNA (aDNA) during the Late Neolithic (11). The spread of farming practices westwards along the northern Mediterranean sea-board is believed to have taken place by ‘punctuated maritime pioneer colonisation’, with subsequent adoption of agrarian practices by indigenous populations (12-14). As the material cultures (12) and the system of management of the animal resources (19) are very diverse within the Early Neolithic communities of the North Mediterranean area, we hypothesise that dairying and dairying practices might have varied much from one region to another.

Here we synthesise new and published evidence to produce a broad regional and chronological perspective on domestic animal exploitation during the 7<sup>th</sup> to 5<sup>th</sup> millennium BC across the Northern Mediterranean and Anatolia (Fig. 1). We specifically examine whether dairying arose in response to particular environmental characteristics or whether it was driven by cultural traditions introduced in the Neolithic. The results of new lipid residue analyses carried out on 567 sherds from this study are combined with previously published results from

the eastern Mediterranean basin (9, 20-22, 23; Table SI1). Lipids were analysed using chromatographic, spectrometric and isotopic methods to characterise their source and identify dairy and carcass residues. Osteo-archaeological age at death (AtD) data for cattle and caprines were collected and mortality profiles were assessed using correspondence analyses (CA) bi-plots, in order to assess slaughter practices (Table SI2). These complementary data sets are combined to provide a comprehensive regional perspective of prehistoric animal exploitation.

## **Results**

### ***Overview of biomarker and isotopic analyses***

Interpretable residues (>5 µg of lipids per g of sherd) were extracted from only 27% ( $n = 153$ ) of the 567 vessels analysed (Table S1); a frequency of preservation consistent with previous studies of eastern Mediterranean prehistoric pottery (9). In most cases, molecular compositions of total lipid extracts were consistent with degraded animal fats, with C<sub>16:0</sub> and C<sub>18:0</sub> fatty acids generally predominating the lipid assemblage, while the presence of branched chain fatty acids (C<sub>15:0</sub> and C<sub>17:0</sub>) supported a ruminant origin (24). Ninety-eight potsherds produced sufficient concentrations of *n*-alkanoic acids (C<sub>16:0</sub> and C<sub>18:0</sub>) for determination of their δ<sup>13</sup>C values by GC-C-IRMS (Table S3). The δ<sup>13</sup>C values of the C<sub>16:0</sub> and C<sub>18:0</sub> fatty acids reflect their biosynthetic and dietary origin, allowing non-ruminant and ruminant adipose and ruminant dairy fats to be distinguished (25, 26, 27; Fig. 2).

### ***The Levant and Anatolia, a review of published organic residue analyses and AtD studies***

Dairy ruminants were originally domesticated (1, 28) in this region where European Neolithic cultures originated (16). Caprines dominated Pre-Pottery Neolithic B (PPNB) and PN (Pottery Neolithic) assemblages (29); previous analysis has indicated that caprine dairy management was practised during the PPNB whereas during the Pottery Neolithic (PN) periods, there was a development towards mixed subsistence practices (3). Indeed, PN Near Eastern sites display herd structures dominated by adult animals of prime meat age (Fig. 3c). In the absence of ceramics, there is of course no lipid data for the PPN, but lipids were only detected in around 10% of the PN sherds from sites investigated (second half of the 7<sup>th</sup> to 6<sup>th</sup> millennium BC: Tell Sabi Abyad (23), Sha'ar Hogolan: (9); al-Basatîn: (21)). For example, at Tell Sabi Abyad around 11% of the sherds contained animal fats of which 13% were dairy in origin (Fig. 2a-b). Ruminants were managed for numerous products, and the use of non-ceramic containers for milk processing (22) could explain the apparent low frequency of dairy residues in ceramic

pots from the region.

In central and western Anatolia, caprines dominate faunal assemblages (30) and the identification of dairy husbandry from AtD is hampered by the lack of published information for both caprines (3 sites) and cattle (1 site). However, previous faunal assessments and our CA suggest that caprines were managed for dairy (Fig. 3c-d; Erbaba Höyük: (31); Ulucak Höyük: (32)). Milk use was not particularly evident at Çatalhöyük, where only 8% of the animal fats detected were of dairy origin ((9); Fig. 1, 2a-b). The analysis of post-cranial AtD from the site suggests that cattle were slaughtered after 24 months (30), and if cattle were managed for milk, it would have been shared between the herders and the growing calf (33). In contrast, extensive sampling of potsherds ( $n = 537$ ; 6 sites) around the Sea of Marmara revealed that milk was used extensively in the area from the second half of the 7<sup>th</sup> millennium BC (9), as more than 70% of the animal fats extracted were identified as dairy fats (Fig. 2c). This coincides with an increase in cattle herds in the region (9), although there is growing evidence of the important role of caprines as milk producers (32). Since cattle dental remains are highly fragmented it is difficult to assess whether they were the main dairy producers in this region (34).

### ***Northern Greece and Aegean seaboard***

Neolithisation of Greece is thought to have happened (i) by land from NE Anatolia to Thrace and the Balkans and/or (ii) by sea from the Aegean Anatolian coast or the Levantine coast (35-38). Lipid residues characterised from 421 potsherds (116 sherds from this study; 305 sherds from ref. 9) from 6 Middle and Late Neolithic northern Greek sites dating to the 6<sup>th</sup>-5<sup>th</sup> Millennium BC showed that less than 10% of the sherds with animal fats contained dairy fats (Fig. 2d). However, the potential processing of pig products, suggested by the presence of extensive pig remains at the sites, could have prevented identification of milk residues in pots, since mixtures of porcine and dairy fats have similar  $\Delta^{13}\text{C}$  values as ruminant adipose fats. Nevertheless, the low incidence of dairy fats in pottery is echoed by the results from the faunal analysis, as both the caprine and cattle CA (Fig. 3) show that meat was the main focus. The primary meat exploitation is consistent with previous faunal research, which has demonstrated its important role in the Early Neolithic societies (38-40). Neolithic settlements on the smaller Aegean islands were not established until the end of the Greek Middle Neolithic (~5300 BC), probably due to the need for communities to adapt to the inhospitable nature of the islands, i.e. in terms of poor water supply and lack of forest cover (41). These

communities relied more on caprines compared with mainland sites, due to the adaptability of caprines to marginal landscapes (41, 42). The Aegean island sites (Kalythine cave, Rhodes; Ftelia, Mykonos) are characterised by an abundance of caprines of young age classes associated with dairy husbandry (Fig. 3c), which would have provided Neolithic communities with an important protein source in a marginal environment. To our knowledge, the archaeozoological evidence is the sole proxy currently available for milk exploitation in this region.

### ***Adriatic / Central Mediterranean regions (Slovenia, Malta, Croatia and Italy)***

The first *Impressa* Ware culture was identified in the Adriatic region around 6,000 cal. BC, introduced together with domesticates by pioneer sea-faring farming communities (17, 43). Archaeozoological analyses suggest that both caprines and cattle were managed for milk, with specialised intensive husbandries for the former (4, 19, 44, 45). Ages at death for caprines from *Impressa* sites group around the post-lactation, prime meat and adult classes suggesting mixed husbandries, possibly including milk production (Fig. 3c-d). Cattle were intensively slaughtered during infancy and post-lactation, probably associated with dairying (Fig. 3a-b; 45). Analyses carried out on 189 *Impressa*/EN sherds collected from 14 early farming sites from the region (including 36 sherds from ref. 20), identified dairy residues in almost half of the sherds containing animal fats, indicating a high prevalence for the use of dairy products (Fig. 2e). Both lipid residues and archaeozoological information thus provide complementary evidence for milk exploitation in this region during the 7<sup>th</sup> to 5<sup>th</sup> millennium BC.

### ***Southern France and the Iberian Peninsula***

The first Neolithic settlements in southern France appear during the first half of the 6<sup>th</sup> millennium BC and are associated with the Italian *Impressa* culture, with the distinctive Franco-Iberian Cardial tradition developing in the second half of the 6<sup>th</sup> millennium BC (46). Cave and open-air sites appear to play contemporary roles in husbandry strategies, with caprines dominating archaeozoological assemblages (19, 47). Ages at death of caprines for open-air sites are centred close to prime meat production age classes (1-4 years; Fig. 3c-d) whereas cave sites are closely associated with young age classes related to dairy production. For the cattle CA, sites cluster between infant, post-lactation, and prime meat age classes, with a trend towards dairy husbandry in open-air sites (Fig. 3a-b). A third of the sherds analysed from rock-shelters and caves in southern France and the Iberian Peninsula (Grotte Gazel, Font Juvénel and Can Sadurní) contained animal fat residues of which 60% were dairy

in origin (Fig. 2f), correlating with the findings of the archaeozoological study. To date, no sherds from open-air sites from this region have yielded lipid residues. Rock-shelters and caves provide natural stalls that would have been ideal as birthing stations and dairies, and would have offered shelter for herds using areas for alternative grazing pastures, integral to the stock herding seasonal cycle (48). However, open-air sites would have been permanently occupied, and perhaps lactating females would have been kept at those sites year-round.

### ***Statistical analysis of the data set***

Statistical analyses were carried out to assess the correlation between the presence/absence of evidence for dairying (based on faunal mortality evidence and/or presence of dairy lipids), and Köppen-Geiger climate type (49), altitude, site location (coastal/inland) and ceramic cultural affiliations. The dataset contains 82 sites dating from the 8<sup>th</sup>-5<sup>th</sup> millennium BC; evidence for dairy is based on the ORA and AtD data (Fig. 1, Table S4). The variables that were statistically significant using ANOVA were *region* (ANOVA, DF=6, F=6.69,  $p<0.001$ ), *site type* (ANOVA, DF=3, F=5.09,  $p<0.001$ ) and *cultural affiliation* (ANOVA, DF=5, F=5.64,  $p<0.001$ ; Table S5). **Further tests demonstrated that there was a significant presence of dairy activities in the regions of the PPNB, PN of the Marmara region and *Impressa*/Cardial ware cultures compared to Northern Greece (Fig. 1, 4).**

The Köppen-Geiger codes used to define the climate regions were not found to be very significant (ANOVA, DF=6, F=2.1,  $p=0.05$ ), nor were groupings based on overall climate type, precipitation and temperature. Previous research has also shown this lack of correlation between prehistoric faunal evidence and modern climatic data (50). Around 6,200 BC, the Mediterranean basin witnessed serious climatic fluctuations and therefore modern proxies may not adequately define prehistoric climates (51). The correlation between caprine dairying and cave sites obtained for the *Impressa*/Cardial ware communities results from a partial seasonal mobility from plain to the cave hill sites, as an adaptation to the rugged terrain of France and the Iberian Peninsula. In contrast, well-watered open landscapes such as southern Italy and northwestern Spain appear more suitable for specialised cattle dairy husbandry (45). Consequently, the influence of the external environment cannot be dismissed; however, better climate proxies are needed to test this.

### **Discussion**

The early PPN communities of the Levant and Anatolia managed caprines for dairy products (3, 4) and ceramic vessels were used to process milk from the very beginning of pottery



production, as it is evident in the Sea of Marmara region (9). However, in Europe milk exploitation varied from East to West along the northern Mediterranean seaboard, as seen in the quasi-absence of dairy residues in ceramic vessels from northern Greece, in contrast to the strong evidence for dairying in the northwestern Mediterranean. The former cannot be solely explained by the potential use of perishable containers for milk processing or mixing with porcine fats, because age at death profiles have shown that husbandry was focused on meat production in these communities. Moving westwards, osteo-archaeological age at death profiles and lipid residue findings strongly demonstrated that early Neolithic communities were both actively managing animals for milk and processing milk in ceramic vessels (Fig. 1). Combined evidence from faunal and lipid residue analyses therefore unequivocally show that the production and use of dairy products was widespread across the breadth of the northern Mediterranean except in mainland Greece, from the onset of agriculture. Milk and dairy products might have been an important staple in early farming communities, and one of the key drivers in the spread and maybe in the adoption of animal domestication (2).

It has been proposed that environmental factors play an important role in the observed differences in Early Neolithic faunal abundances, more so than the cultural context (50). Indeed the choice of dairy animals would have been heavily influenced by the external environment as it is crucial to the growth and stability of dairy herds. However, from our analysis, we also suggest that the cultural context could possibly also have influenced whether or not dairying was practised, as seen in the difference between northern Greek communities and the wider Mediterranean seaboard. This should be tested further using well-defined geographical and ecological models that reflect prehistoric environments. These data need also to be incorporated into milk production models to generate new approaches to examining the evolution of domestic animal herds across different regions and within cultural groups. The observed differences in the frequency of dairy versus non-dairy exploitation between contemporary groups in Europe during the 7<sup>th</sup>-5<sup>th</sup> millennium BC is intriguing and may be the result from different cultural traditions, environments or dairying abilities of the ruminant lineages.

## **Materials and Methods**

***Organic residue analysis.*** For this study, a total of 567 potsherds were sampled from 21 Neolithic and Chalcolithic sites across the Mediterranean area (Fig. 1; Table S1). Lipid analysis and interpretations were performed using established protocols described in detail in earlier publications (52, 53). Briefly, ~2 g of potsherd were sampled following cleaning of the vessel surfaces with a modelling drill to remove any exogenous lipids. Powdered sherds were solvent extracted by ultrasonication. Aliquots of the total lipid extract (TLE) were trimethylsilylated using *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and submitted for analysis by gas chromatography (GC) and GC-mass spectrometry. Further aliquots of the TLE were hydrolysed and methylated to obtain fatty acid methyl esters (FAMES). FAMES were then analysed by GC and GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS). Instrument precision was  $\pm 0.3\%$ .

***Age at death data collection and processing.*** Osteo-archaeological age at death (AtD) data were collected from ruminant mandibles and isolated teeth from well-dated sites, where sampling strategies focused on defined contexts. Correspondence analysis (CA) bi-plots were used to elucidate trends in the data and generate hypotheses concerning slaughter practices (3). This was performed on cattle and caprine AtD frequencies collected from published reports comprising 50 sites from the study regions dating between 7<sup>th</sup>-5<sup>th</sup> millennium BC (Fig. 3; Table S2). The open access CA program as described in Nenadić and Greenacre (54) for R program (V2.15.2) was used to process the AtD and plots row and column points representing individual site AtD frequencies and age classes, respectively, as two data clouds on the same bi-plot. The position of the individual sites relative to the age classes indicates the dominant slaughter strategy, allowing the overall husbandry strategies practiced to be proposed.

***Statistical analysis.*** A suite of statistical analyses (ANOVA; Chi-squared; Kruskal-Wallis) were carried out on a data set comprising the presence/absence of evidence for dairying, which includes Köppen-Geiger climate type (49), site type, altitude, region and cultural affiliation (Table S3). These were carried out using the R program (V2.15.2).

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### **Author contributions**

C.D.S., R.E.G. and M.R.-S. contributed equally to this work. C.D.S., R.E.G., M.R.-S., O.E.C., J.-D.V. and R.P.E. planned the project and wrote the paper. R.E.G. performed the statistical archaeozoological analyses and C.D.S. and M.R.-S. the lipid residue analyses. Statistical analyses of the dataset were performed by R.E.G. and C.D.S. The other co-authors directed sampling of archaeological material, directed excavations, helped with the archaeozoological studies or carried out lipid residue analyses. All authors read and approved the final manuscript.

## Figure captions

**Figure 1.** Map of the Mediterranean basin showing the location of the sites in which organic residue analysis and archaeozoological studies were carried out, including data from the present study and published literature. The ceramic vessels and faunal remains tested date to the 7<sup>th</sup>-5<sup>th</sup> millennium BC. The map highlights the geographical evidence of dairying during this time. [1: Shiqmin, 2: Al-Basatín, 3: Sha'ar Hagolan, 4: Aswad, 5: El Kown 2 (lower levels), 6: Qdeir, 7: Umm el Tlell, 8: Seker (PN), 9: Sotto, 10: Çayönü Tepesi, 11: Tell Sabi Abyad, 12: Akarçay Tepe, 13: Halula 25, 14: Halula 26, 15: Mezraa Teleitai, 16: Domuz Tepe, 17: Tepecik Çiftlik, 18: Shillourokambos, 19: Çatalhöyük, 20: Er Baba Höyük, 21: Suberde, 22: Hoyucek, 23: Knossos, 24: Ftelia, 25: Lerna, 26: Kalythies cave, 27: Ulucak Höyük, 28: Barcin Höyük, 29: Hoca Çesme, 30: Yarimburgaz, 31: Toptepe, 32: Pendik, 33: Fikir Tepe, 34: Aşagi Pinar, 35: Makri, 36: Sitagroi, 37: Stavroupoli, 38: Paliambela, 39: Makriyalos, 40: Prodrornos, 41: Dispilio, 42: Ritini, 43: Toumba Kremastis Koiladas, 44: Apsalos, 45: Nakovana Cave, 46: Pupincina, 47: Mala Triglavca, 48: caves of Trieste Karst (Edera, Mitero, Zingari), 49: Masseria La Quercia, 50: Canne - Sette Ponti, 51: Palata 1, 52: Trani - Seconda Spiaggia di Colonna, 53: Fondo Azzollini, Pulo di Molfetta, 54: Serri - San Gabriele, Bari San Paolo, 55: Masseria Maselli, 56: Balsignano, 57: Ciccotto, 58: Trasano, 59: Torre Sabea, 60: Grotta San Michele, 61: Favella della Corte, Corigliano Calabro, 62: Skorba, 63: Colle Santo Stefano, 64: La Marmotta, 65: Araguina-Sennola, 66: Arene Candide, 67: Grotte Lombard, 68: Baume de Fontbrégoua, 69: Abri II du Fraischamp, 70: Abri de Saint-Mitre, 71: Barret de Lioure, 72: Combe Obscure, 73: Baume d'Oullen, 74: Pont de Roque-Haute, 75: Grotte Gazel, 76: Font-Juvénal, 77: Abri Jean Cros, 78: Can Sadurní, 79: La Draga, 80: Cova de Chaves II, 81: Caserna de Sant Pau, 82: Cova de la Sarsa, 83: Los Castillejos, 84: Cueva de Nerja]. Dating of the sites can be found in Table S6.

**Figure 2.**  $\Delta^{13}\text{C}$  values for archaeological animal fat residues in Neolithic pottery from (a) The Levant (9, 21), 3 sites; (b) Central and eastern Anatolia (9), 8 sites; (c) Northwestern Anatolia (around the sea of Marmara; (9)), 7 sites; (d) Northern Greece (this study and (9)), 6 sites; (e) Italy, Slovenia, Croatia and Malta (this study and (20)); 8 sites and (f) Southwestern France and Spain (this study); 3 sites. The ranges shown here represent the mean  $\pm$  1 standard deviation of the  $\Delta^{13}\text{C}$  values for a global database comprising modern reference animal fats (26).

**Figure 3.** F1 x F2 biplot correspondence analysis (CA) for cattle, based on (a) the minimum number of individuals (MNI) and 15 contexts; and (b) the number (Nd) of dental fragments and 9 contexts; and sheep/goats based on (c) MNI and 20 contexts and (d) Nd and 45 contexts, respectively. CA plots were constructed using dental fragments analyses for 43 sites from Anatolia (PN sites; green); Near East (PN sites from: Syria and Iraq dark blue); Greece (EN-LN, 8<sup>th</sup>-6<sup>th</sup> millennium BC: dark grey); Italy and Croatia (*Impressa*, EN, 7<sup>th</sup>-6<sup>th</sup> millennium BC: yellow); Southwestern France and Spain (Cardial, EN, 7<sup>th</sup>-6<sup>th</sup> millennium BC: light blue), Open circles: cave and rock shelter sites; Closed circles: open air and tell sites. The triangles represent the age classes, and their size reflects the influence on the data. For caprines: age class A: 0-2 months, B: 2-6 months, C: 6-12 months, D: 1-2 years, EF: 2-4 years, G: 4-6 years, HI: + 6 years. Sites that are positioned close or between infant/juvenile age classes (cattle: 0-12 months; caprines: 0-6 months) and mature adults (4+ years) could be an indication that dairy husbandry was practised. Numeration for the sites as in Figure 1.

**Figure 4.** Bar charts for the presence (white) and absence (dark grey) of dairying for (a) site types (ANOVA, DF = 3, F = 5.09, p<0.001), (b) cultural groups (with ICW: *Impressa*/Cardial ware, PNG: Pottery Neolithic Greece, PNM: Pottery Neolithic Marmara, PNA: Pottery Neolithic Anatolia, PNL: Pottery Neolithic Levant, PPNB: pre-pottery Neolithic B; ANOVA, DF = 5, F = 5.64, p<0.001), (c) climate types (abbreviations according to Köppen-Geiger climate types (49); ANOVA, DF = 6, F = 2.1, p=0.05) and (d) regions (ANOVA, DF = 6; F = 6.69, p<0.001. See Tables S4-5 for complete dataset.