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Population dynamics, cultural evolution and climate change in pre-Columbian western South America

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Abstract
This paper reports on an archaeogenetic study that was embedded in a transdisciplinary research project with the principal aim of understanding the cultural development in pre-Columbian Southern Peru. Ancient DNA analyses were conducted on over 300 pre-Columbian individuals from various archaeological sites in coastal Southern Peru and the Andean highlands, from periods ranging from the middle Formative Period (approx. 1000 BC) to the end of the Late Intermediate Period (approx. AD 1400). The data obtained were compared against a large set of data on contemporary and ancient South American populations to reveal biological affinities across the southern Andes and, more broadly, on the continental level, and contextualized with the archaeological and palaeoecological record.

The results, albeit preliminary, shed light on population-dynamic processes accompanying cultural transformations in several archaeological periods. Changes in socioeconomic complexity and ecological alterations seem to have influenced migrational behaviour and the genetic diversity of the prehistoric central Andean populations. On the continental scale the results point to a discrete initial peopling of the western South American coast and the Andean highlands.

Keywords
Ancient DNA, Nasca, Paracas, population genetics, mitochondrial DNA, cultural change

Introduction

Central western South America offers the perfect conditions for the study of the reciprocal effects of cultural, ecological and population-dynamic processes. The pre-Columbian cultural landscape is characterized by two adjacent main cultural areas with extremely different ecological conditions: the Pacific Coast, including the western slopes of the Andes, and the Andean Highlands. Within a time span of approximately 13,000 years (Dixon, 2001; Dillehay, 2009) the two areas witness the transition from small nomadic groups to sedentism, the evolution of complex societies and even states. These developments are the results of complex patterns of interaction between the two areas, with constantly altering reciprocal interferences, and changes in the intensity and direction of cultural transmission. To what extent these cultural processes were accompanied or influenced by population-dynamic processes and alterations of mobility patterns and even gene flow is a question that remains for the most part unanswered.

I report on an archaeogenetic study that was embedded in a transdisciplinary research project with the principal aim of understanding the cultural development in pre-Columbian Southern Peru. The research area forms a transect of about 100 km spanning from the Pacific coast to the highlands, with levels of elevation ranging from 0 to 4500 above sea level, and thus traversing the main cultural areas and a multitude of different ecological zones. Ancient DNA analyses were conducted on over 300 pre-Columbian individuals from various cemeteries in the Palpa area, the Rio Grande estuary, the Paracas Peninsula, and the adjacent Andean highlands (Fig. 1), from periods ranging from the Formative Period to the Late Intermediate Period (LIP) to reveal the population dynamics in this settlement chamber in a timeframe of approx. 2,200 years. The data obtained were compared against a large dataset on contemporary and ancient South American populations to reveal biological affinities across the Andes and,
more broadly, on the continental level, and contextualized with the rich basis of archaeological and ecological data from our transdisciplinary research project.

The following two sections will give a brief introduction to the genetics of indigenous South American populations and the observations of archaeology and palaeoecology.

**The indigenous population of South America**

Despite the richness of their cultures and the richness of the environments that they inhabit, the Native Americans harbour a relatively low level of genetic diversity. Nearly all Native American mtDNA haplotypes belong to one of four ancestral lineages, the mt haplogroups designated A, B, C, and D (Torroni et al., 1993). These lineages are widely found throughout the Americas, but there is a lot of variation in frequencies among populations and geographic regions. A fifth founding mitochondrial haplogroup, des-
ignated X, is found only in indigenous populations of northern North America (Dornelles et al., 2005). All of those five major matrilineages (mt haplogroups) were represented by only one (Schurr and Sherry, 2004) or a few (Kemp et al., 2007; Tamm et al., 2007; Perego et al., 2009; Malhi et al., 2010) sublineages (mt haplotypes) in the initial founding population. The mt haplogroups are definitely of Asian ancestry, and the genetic data indicate that the ancestral source population probably originated in south-central Siberia, from whence it migrated to Beringia and then into the New World (Schurr and Sherry, 2004).

According to the current state of knowledge, the most parsimonious model from genetic data features a single initial migration with relatively few individuals about 20,000–14,000 years ago along a Pacific coastal route (Merriwether et al., 1995; Schurr and Sherry, 2004; Lewis et al., 2007a) and maybe a second wave later following the ice-free corridor (Perego et al., 2009), maintaining recurrent gene flow with Asia following the initial peopling (Ray et al., 2010). With respect to Y-chromosomal DNA, most male Native Americans belong to one of the two principal founding lineages C and Q (nomenclature: Y-Chromosome Consortium 2002). The haplogroup Q1a3a* (formerly Q-M3) is the most frequent in Native South American males, at 77% (Bortolini et al., 2003; Karafet et al., 2008).

There is compelling archaeological and genetic evidence to suggest that South America had been peopled by 14–13,000 BP (Dillehay, 1999; Fuselli et al., 2003). Recent genetic comparison of modern Native American populations from all over South America show that although there are regional differences in the patterns of genetic variation, the low overall variance among these regions gives no evidence for several migrational waves (Lewis et al., 2007a; Lewis, Jr., 2009). Although this most parsimonious interpretation is therefore that the continent was peopled by one founding population, the exact routes they followed, and whether the wave split into different groups when passing the Isthmus of Panama, remain unknown (Lalueza et al., 1997; Keyeux et al., 2002; Lewis et al., 2007b). In the past, the classic assumption was that eastern and western South America show different grades of genetic diversity (Fuselli et al., 2003; Lewis, Jr. et al., 2005; Lewis, Jr. and Long, 2008). Although newer studies based on autosomal DNA have proven that diversity in the two regions is comparable and that previous studies suffered from sampling biases (Lewis, J.R., 2009), the complex patterns of diversity observed are far from being understood. There is a very specific regional distribution of mitochondrial haplogroup frequencies (Fig. 2) and a high frequency of mt haplotypes that are unique and not shared among different regions. When concentrating on western South America there is a gradient with high frequencies of Haplogroup A in the north-west, followed by the predominance of Haplogroup B throughout the Central Andean area, and D in southernmost South America.

The number of palaeogenetic studies conducted for South America is still small. Only recently has there been an increased interest in palaeogenetic studies of pre-Columbian Central Andean populations from the coast (Shimada et al., 2004; Moraga et al., 2005; Fehren-Schmitz et al., 2009; Fehren-Schmitz et al., 2010) and the highlands (Shinoda et al., 2006; Lewis et al., 2007a; Kemp et al., 2009; Carnese et al., 2009; Fehren-Schmitz et al., 2011).

Archaeology and ecology of the Palpa region

Beginning with the Middle Archaic Period (3800 BC), the archaeological record in the Palpa region provides evidence of a nearly continuous occupation until the Late Horizon (AD 1400–1532), represented by the Inca culture. The earliest definitely permanent settlement structures date to the Initial Period (1800–800 BC). With the Early Horizon (800–200 BC), there is a continuous increase in the number of
Fig. 2 | Gradient map showing the continental mt-haplogroup frequency distribution in South America. The white line indicates the major expansion of the Inca Empire. The map is based on haplogroup data on 198 modern indigenous populations (Fehren-Schmitz, 2008) and was drawn using MapViewer™, employing the Kriging mode of interpolation.
sites in the Palpa region and, with that, in settlement density. The Early Horizon of the Peruvian south coast is characterized by the Paracas culture (Tello, 1959) described by the ceramic inventories from the Ica Valley (Menzel et al., 1964) about 80 km north of Palpa. It has been possible to attest to the presence of the Paracas culture in the region over the whole Early Horizon through archaeological evidence from excavations and field surveys in the Palpa valleys (Reindel and Isla-Cuadrado, 2003; Isla Cuadrado and Reindel, 2003; Reindel et al., 2004; Reindel and Isla-Cuadrado, 2006), challenging the earlier hypothesis that the spatial extension of the Paracas culture did not reach the Rio Grande de Nasca until the late phases (Silverman, 1994). The detection of Paracas sites in the northern heartlands of the Nasca culture contravenes previously formulated hypotheses of Nasca origin. It is commonly stated that the Nasca tradition evolves out of the Paracas culture (Menzel et al., 1964; Silverman and Proulx, 2002). The fact that, until recently, there was little to no evidence for Paracas occupation in the main territory of the Nasca culture (Orefici, 1996; Schreiber and Lancho Rojas, 2003; Vaughn and Gijseghem, 2007) was interpreted as evidence that the Nasca culture did not emerge autochthonously in the Rio Grande drainage. It was postulated that in the Late Paracas period people migrated from the Ica area into the Rio Grande de Nasca drainage, where they accounted for the formation of the Proto Nasca culture (ca. 200 BC–AD 60 [Silverman, 1994]). The archaeological evidence from the Palpa region, along with the discovery of petroglyphs and geoglyphs in the Palpa area dating to the Early Horizon, was interpreted by Reindel et al. (2004) as proving that the Nasca culture developed autochthonously in the Rio Grande drainage.

Palaeoecologic data show that both the flourishing of the early Nasca and the development of irrigation systems correlate with increasing aridity and slight aggravation of the ecological conditions for the inhabitants of the south coast valleys (Eitel et al., 2005). A great number of sites have been detected for the Early and Middle Nasca phases. In addition to numerous rural settlements, two large (urban) settlement centres, with planned layout, central buildings and other characteristics of formal settlements, have been excavated in the Palpa valleys (Reindel and Isla-Cuadrado, 2001). The multilevel hierarchy settlement patterns observed, the complex cultural landscape, and the large elite burials encountered in La Muña indicated that the Nasca reached a far higher grade of political organization than had previously been assumed (Isla-Cuadrado and Reindel, 2006).

During the Late Nasca Period (A.D. 430–600) the number of sites in the Palpa region starts to decrease. Previous hypotheses assumed that the decline of the Nasca culture may have been caused by the militaristic expansion of the Wari from the Central Andean highlands at the beginning of the Middle Horizon (Allison, 1979). There is no evidence in the archaeological record in Palpa for an increased foreign impact or warlike activities that would justify the assumption of invasion or elite dominance scenarios. In the Middle Horizon (A.D. 600–1000) the Palpa region was nearly abandoned (Reindel and Isla-Cuadrado, 2000). The palaeoecologic data show that with the Late Nasca period came a major increase in aridity, causing the desert margin to shift eastward. Based on these observations, Eitel and Mächtle (2006) postulate that the end of Nasca was caused by the deterioration of ecological conditions and desertification of the settlement area.

This situation changed again around AD 1000 when climatic conditions became more humid (Eitel and Mächtle, 2009) and population density increased considerably. People concentrated in large habitation sites like Ciudad Perdida and population numbers must have equalled those of Early Nasca times. Beginning in 1400, as the prehispanic period drew to a close, climatic conditions again became dryer and the number of settlements in the area decreased to a minimum (Reindel and Gruen, 2006).
Materials and methods

Samples

Bone and teeth samples from 350 skeletons or mummified human individuals were collected in several field campaigns (2004–2009). Of those, 193 were from various archaeological sites in the Andean foothills (Chala ecological zone) around the modern city of Palpa. The Palpa samples were collected from chronologically varying sites, allowing the investigation of a timeframe stretching from the Formative period (approx. 1000 BC) to the Late Intermediate Period (LIP: AD 1000–1400). Sites with burials from more than one archaeological phase were preferred, in order to reveal population continuities or discontinuities. An additional 90 individuals were sampled from sites dating to the MH and LIP located in the upper valleys of the Rio Palpa and Rio Viscas, directly adjacent to the Andean Puna at an altitude of approximately 3000–4000 m – Quechua and Suni ecological zones (Pulgar-Vidal, 1979) – in the environs of the modern town of Laramate, 50 km to the east of Palpa. Forty individuals (EIP and LIP) derive from the coastal site Monte Grande, located at the Rio Grande estuary, and additionally, 12 individuals derive from the Early Horizon coastal site Paracas Caverna 6, Paracas Peninsula, 150 km north of Palpa. Sampled sites, their chronological classifications, and the number of sampled individuals sampled from each site for each chronological period are listed in Table 1. All samples were acquired under strict observance of the established precautions for ancient DNA studies (Bollongino et al., 2008). Material was immediately sealed to prevent contamination and documented anthropologically before being exported to our laboratories in Germany with the permission of the Institute of Peruvian Cultural Heritage. The preparation of the bone and tooth samples for DNA extraction followed a standardized protocol (Hummel, 2003) and was performed in facilities dedicated solely to ancient DNA research (SAM-LAB).
extractions were conducted utilizing a new protocol combining complete demineralization (Loreille et al., 2007), DNA binding to silica (Rohl and Hofreiter, 2007), and automated purification (Fehren-Schmitz et al., 2010). Further information on the methods and protocols used to extract DNA from the ancient specimens are described in Fehren-Schmitz et al. (2010, 2011).

Additionally, published comparative haplogroup frequency and sequence data from over 5000 individuals of modern and ancient Native South American populations were obtained. A complete listing of all populations considered has been published by the present author (Fehren-Schmitz, 2008).

**Genetic markers**

The studies presented here were mainly based on the analysis of mtDNA, and hence of matrilinear population dynamics. Further investigations concerning y-chromosomal binary markers (y-chromosomal haplogroups; Fehren-Schmitz et al., 2011), autosomal STRs (genetic fingerprints) and chromosomal markers associated with birth weight, adaptation to highland habitats and other issues of anthropological genetics are still in process.

To type the mitochondrial haplotypes, we analyzed a 388 bp fragment of the mitochondrial HVR I (nucleotide position 16021–16408 relative to the revised Cambridge reference sequence (rCRS) [Andrews et al., 1999]) employing an analysis system consisting of eight primers generating overlapping PCR products. The generated PCR products were further analyzed by direct sequencing. For information regarding analysis parameters, protocols, primer sequences and PCR conditions see the work of Fehren-Schmitz and colleagues (2010). In addition to the analysis of the mitochondrial haplogroups based on specific HVR I polymorphisms, we analyzed four specific polymorphisms of the mitochondrial coding region, determining the groups A, B, C, and D (Fig. 2). Three of the groups – A, C, and D – are characterized by SNPs (single base transversions or transitions) and Group B is characterized through a 9 bp deletion at nucleotide position (np) 8272–8280 of the mitochondrial genome (Merriwether et al., 1995). Additional polymorphisms determining the main groups M, N, R, L3 were typed together with the four polymorphisms described above in a multiplex SBE assay to discriminate between possible contaminating sequences and authentic sequences (Fehren-Schmitz et al., 2011). This twofold analysis system for mt-haplogroups was developed to authenticate results due to the high risk of false determinations associated with the possibility of rapidly evolving mutational hotspots at the HVR I and miscoding lesions caused by postmortem DNA damage (Meyer et al., 1999; Willerslev and Cooper, 2005; Gilbert et al., 2007). Both methods were used on the same DNA extracts. For every sample there were at least two independent extracts. For further information refer to Fehren-Schmitz et al. (2011).

Standard genetic diversity indices, interpopulation distances, population structure, and other population genetic standard parameters were calculated for all populations analyzed, employing the Arlequin software package (version 3.1, Excoffier et al., 2005). Exact tests of haplogroup and haplotype frequency differences were also conducted for all populations (Schneider and Excoffier, 1999). Similarities between populations and across time periods were graphically assessed using Principal Component Analysis (PCA), and Multi Dimensional Scaling (MDS), and distance-based phylogenetic trees and MJ-networks from sequence data were calculated using the Mega 4.0 software (Tamura et al., 2007) and Network 4.5 (Bandelt et al., 1999). Further information regarding the population genetic analyses and detailed results have already been published (Fehren-Schmitz, 2008; Fehren-Schmitz et al., 2009; Fehren-Schmitz et al., 2010; Fehren-Schmitz et al., 2011).
Results

To date it has been possible to reproducibly determine the mt haplogroups of 220 individuals (63%), through the analysis of coding region polymorphisms, and to obtain the mt haplotypes (complete 388 bp HVR I sequence) of 201 individuals (57%) from a total of 350 individuals sampled in the project. The successfully analyzed samples represent the entire range of archaeological sites and periods that have been sampled (cf. Table 1). Amplification of chromosomal DNA has thus far been successful for 25 individuals (Fehren-Schmitz et al., 2011; Fehren-Schmitz, 2008), most of whom were from the highland sites, but the chromosomal investigations have only just begun and all results are preliminary.

Generally it can be stated that the quality of DNA preservation found in the individuals from the highland sites proved better than that in the individuals from desert burials. The burial environment encountered in the chullpas and burial caves, where most of the highland samples were collected, is characterized by a stable level of humidity and temperature. The bones in such sites have only little soil contact. These conditions have proven to be very good for DNA preservation in other studies (Burger et al., 1999). The poorer DNA preservation in the Palpa area may be explained in part by higher temperature and soil pH-value, but it may also be due to the changes in environmental conditions over time (Eitel and Mächtle, 2006) and the associated changes in storage conditions of the region.

All successfully typed individuals belong to one of the four Native American haplogroups A, B, C and D, with the findings independently confirmed through the coding region polymorphisms and HVR I sequences. The haplogroup distribution of the studied pre-Columbian populations, grouped by prov-
Haplogroup D (60–70 %) is predominant in the coastal Paracas and rural Nasca populations, followed by Haplogroup C. Haplogroup B, predominant in the MH and LIP highland populations (55–57 %), first appears in the coastal populations in the EIP with low frequencies (11–18 %). High frequencies of the groups D and C persist in the coastal populations into the MH but in the LIP the observed mitochondrial haplogroup distribution in the coastal populations (coast and Palpa area) changes completely, now exhibiting high frequencies of Group B (50–60 %), rendering it similar to the highland populations in that respect. The haplogroup frequencies of the ancient populations under study were compared to a large dataset on other ancient and modern Native South American populations. Comparison results are displayed in a PCA plot based on pairwise F_{ST} derived from frequency data (Fig. 4). The populations clearly group into two clusters separated by the first PCA. One cluster is made up of the ancient EH to MH Peruvian south coast populations, a MH north coast population (Shimada et al., 2004) and the modern...
Table 2 | Mitochondrial haplogroup frequencies and haplotype diversity (HD) for the analyzed pre-Columbian and the ancient and modern Native South American reference populations

<table>
<thead>
<tr>
<th>Group</th>
<th>Population</th>
<th>Date</th>
<th>n(^a)</th>
<th>Haplogroup frequency</th>
<th>HD(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H^b</td>
<td>A</td>
</tr>
<tr>
<td>Ancient coast</td>
<td>South Coast (Peninsula)^1</td>
<td>EH</td>
<td>10 (6)</td>
<td>5</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>South Coast (Palpa)^1</td>
<td>EH</td>
<td>28 (25)</td>
<td>15</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Nasca-Rural (Palpa)^1</td>
<td>EIP</td>
<td>37 (30)</td>
<td>26</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Nasca-Urban (Palpa)^1</td>
<td>EIP</td>
<td>28 (25)</td>
<td>17</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>South Coast (Palpa)^1, 15</td>
<td>MH</td>
<td>11</td>
<td>9</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>South Coast^15</td>
<td>LIP</td>
<td>22 (25)</td>
<td>16</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>North Coast^2</td>
<td>MH</td>
<td>(36)</td>
<td>n.d.</td>
<td>0.19</td>
</tr>
<tr>
<td>Ancient highlands</td>
<td>Ancient Upper Valleys^3</td>
<td>MH</td>
<td>27 (30)</td>
<td>13</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Ancient Upper Valleys^3</td>
<td>LIP</td>
<td>38 (42)</td>
<td>25</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Conchapata^4</td>
<td>MH</td>
<td>10</td>
<td>9</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>Chen Chen^5</td>
<td>MH</td>
<td>(23)</td>
<td>n.d.</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Huairi^4</td>
<td>LIP</td>
<td>17</td>
<td>12</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Paucarcancha^6</td>
<td>LH</td>
<td>(35)</td>
<td>n.d.</td>
<td>0.09</td>
</tr>
<tr>
<td>Northern Colombia</td>
<td>Chibchan^7</td>
<td></td>
<td>80</td>
<td>7</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Arawaken^7</td>
<td></td>
<td>29</td>
<td>4</td>
<td>0.28</td>
</tr>
<tr>
<td>Northern Peru</td>
<td>Ancash^8</td>
<td></td>
<td>33</td>
<td>27</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>San Martin^9</td>
<td></td>
<td>21</td>
<td>14</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Tupe^10</td>
<td></td>
<td>16</td>
<td>9</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Yungay^10</td>
<td></td>
<td>36</td>
<td>20</td>
<td>0.03</td>
</tr>
<tr>
<td>Southern Peru</td>
<td>Arequipa^9</td>
<td></td>
<td>22</td>
<td>18</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Puno (Quechua)^10</td>
<td></td>
<td>30</td>
<td>22</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Puno (Aymara)^10</td>
<td></td>
<td>14</td>
<td>11</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Tayacaja 9</td>
<td></td>
<td>59</td>
<td>40</td>
<td>0.22</td>
</tr>
<tr>
<td>South Middle Chile</td>
<td>Mapuche^11</td>
<td></td>
<td>34 (111)</td>
<td>9</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Pehuenche^11</td>
<td></td>
<td>24 (105)</td>
<td>13</td>
<td>0.03</td>
</tr>
<tr>
<td>Tierra del Fuego</td>
<td>Yaghan^11</td>
<td></td>
<td>15 (21)</td>
<td>7</td>
<td>0.00</td>
</tr>
<tr>
<td>Amazonia</td>
<td>Gaviao^12</td>
<td></td>
<td>27</td>
<td>7</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Xavante^12</td>
<td></td>
<td>25</td>
<td>4</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Yanomami^9</td>
<td></td>
<td>155</td>
<td>6</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Zoro^12</td>
<td></td>
<td>30</td>
<td>9</td>
<td>0.20</td>
</tr>
<tr>
<td>Gran Chaco</td>
<td>Gran Chaco (Pool)^14</td>
<td></td>
<td>204</td>
<td>46</td>
<td>0.16</td>
</tr>
</tbody>
</table>


a  n = Number of individuals with successful haplogroup determination. The number of individuals with successfully determined mt-Haplotypes follows in brackets
b  H = Number of determined different haplotypes in the population
c  Hd = Haplotype diversity (Nei 1987). The value relates to the HVR I sequence data not to the haplogroup data
indigenous populations of southern Middle Chile (e.g. Mapuche, Pehuenche) and Tierra del Fuego. The other cluster consists of the ancient Peruvian highland populations, and modern Central Andean, Amazonian and Gran Chaco populations, plus the LIP populations from the Peruvian south coast. The second PCA mainly distinguishes the northern Colombian population from the rest of the South American populations.

The HVR I sequence data from the 201 successfully typed individuals could be assigned to 106 mitochondrial haplotypes (H). A summary of the analysed mt haplotypes spanning 388 bp of the HVR I (np 16021–16408) and of their distribution over the different sites and chronological periods has been published by the present author and colleagues (2008, 2010, 2011). The Native American founding haplotypes A2, B2, C1 and D1 (Tamm et al., 2007) are the most frequent for every haplogroup. Overall there are 59 singleton haplotypes in the whole dataset. The others are shared synchronously between different sampled archaeological sites or diachronically between periods in the geographic region (Fig. 5). Distinct haplotypes – different from the founding haplotypes – are shared between the coastal EH populations from Palpa and the Peninsula, and the EH, rural and urban EIP, and MH populations in the Palpa area. One individual from the elite burials of La Muna also shares a distinct group C haplotype with two individuals from sites associated with rural settlements. The MH and LIP highland sites also share several
distinct haplotypes synchronously and diachronically. Despite the low geographic distance between the Palpa and the upper valley highland sites the populations share no haplotypes until the LIP. All analyzed ancient Peruvian populations show a high level of genetic diversity (Table 2) congruent with those from other known central Andean populations (cf. Lewis et al., 2007b). Although there is a diachronic and group-specific change in the haplogroup frequencies in the coastal communities, there is no increase of overall mitochondrial genetic variability.

Genetic distance calculations based on the HVR I sequence data support the considerations derived from the haplogroup data. Very low and non-significant distances can be determined between both Paracas period populations and the rural Nasca group. The rural and urban Nasca, and Nasca and Middle Horizon comparisons also yield low and non-significant $F_{ST}$ values (0.053; 0.070). All ancient coastal populations exhibit a high distance to the pre-Columbian highland populations ($F_{ST}$: 0.251–0.354), despite the fact that coastal populations date to the LIP ($F_{ST}$ Coast-Highland LIP: 0.031). In the MDS plot (Fig. 6) the EH, EIP and MH coast populations form a cluster with the populations from Tierra del Fuego, whereas the pre-Columbian Andean highland populations and the Amazonian population pool form a distinct cluster that also includes the coastal populations dating to the LIP (Fig. 6). The modern indigenous populations of Peru cluster close to the latter group. These observations are also supported by AMOVA and other statistical analyses not reported here (cf. Fehren-Schmitz et al., 2011).
Discussion

Population dynamics and cultural evolution in the Palpa Area

When consolidated with the archaeological and ecological knowledge, the genetic data obtained can be translated to reconstruct the population-dynamic processes in pre-Columbian southern coastal Peru. The genetic similarity of the Paracas populations from the Palpa area and the Peninsula, as well as the low genetic distance between them, allows the conclusion that the Paracas period communities in the Ica-Valley, Paracas Peninsula region and the northern Rio Grande de Nasca drainage were not only culturally (Reindel, 2009) but also biologically related. This assumption is supported by the high gene flow detected within the distribution area of the Paracas culture and by the lack of a significant genetic exchange with the surrounding populations, for example, from the Andean highlands (Fehren-Schmitz, 2008, Fehren-Schmitz et al., 2010, 2011). Since the archaeological data and the individuals genetically analyzed in this study also date to the early phases of the Paracas culture (Ocucaje 3–4) it can be assumed that this relationship did not result from a migration out of the Ica area in the late Paracas period (Ocucaje 8–9), as has been suggested by, e.g., Silverman (1994). Distance calculations and distribution patterns also suggest that a constant population in the Palpa area persists into the Nasca period, but of course these assumptions have to be validated by further statistical analysis. These findings, combined with archaeological evidence, such as parallels in ornaments and the existence of early geoglyphs (Reindel and Isla-Cuadrado, 2006; Lambers, 2006), suggest that the Nasca culture of this area probably evolved autochthonously from the bearers of the Paracas culture. Although the urban and rural Nasca populations do exhibit a degree of genetic distance, there is no evidence that that distance results from foreign influences, as one would expect from an elite dominance scenario (Renfrew, 1987). This assumption is supported by the fact that individuals from the elite burials of La Muna share mt haplotypes with the rural populations. The emergence of Haplogroup B and the differences between the rural and urban populations suggest that there is a higher amount of genetic exchange with populations from outside the investigated coast area, maybe the highlands. One must bear in mind that until the LIP the studied coastal and highland populations share no distinct haplotypes other than the founding haplotypes, but the high frequencies of B in the highland populations make them a possible source population for immigration. This might be a result of the increase of socioeconomic complexity in the Nasca period, which would have caused a concurrent augmentation of the migrational pull-factor of this area (Lee, 1972). A grade of social complexity such as that reached in the Nasca culture requires craft and administration specialists (Service, 1962). The fact that the genetic population structure changes slightly in the Nasca period and that the urban Nasca population differs from the rural one might possibly be explained by the immigration of foreign specialists into the urban settlements. There is no significant genetic distance between the Nasca period and Middle Horizon populations from the coast and hence no evidence for population discontinuities in the coastal research area (cf. simulations in Kemp et al. [2009]). The MH populations from the upper valley highland sites and other MH highland populations published previously (Kemp et al., 2009) exhibit significant matrilineal differences from the MH Palpa populations. This observation suggests that invasion or colonization scenarios (cf. Allison, 1979) seem implausible as a cause for the demise of the Nasca culture in this area. The decrease of population density in the Late Nasca period and the Middle Horizon juxtaposed by the constantly high mt haplotype diversity fits best with scenarios suggesting that the carrying capacity of the main settlement area decreases as a consequence of climatic aggravations and their inhabitants emigrate, possibly eastward into
the Andean valleys (Eitel and Mächtle, 2006). But, neither the MH nor the LIP populations of the highland sites studied show any signs of intermixture with the observed coastal genetic signatures. On the contrary, the coastal populations of the LIP exhibit a high affinity to the MH and LIP highland populations and the modern populations of the Central Andean region. The latter phenomenon is best explained by a massive repopulation of the coastal areas in the short humid phase between AD 1000 and 1400 (Eitel and Mächtle, 2009). The genetic homogeneity observed and the evidence of distinct haplotypes shared between both areas make it most likely that the people repopulating the coastal area derived from the adjacent highlands.

One possible model of explanation for the phenomenon that the upper valley populations show no signs of intermixture with the proximate coastal populations that allows for the possibility that there were immigrations from the coast can be based on the effect that physical stressors can have on unadapted humans at altitudes above 2500 m (Fehren-Schmitz et al., 2009). There is evidence from historical sources and high-altitude medicine that the risk of stillbirth is significantly higher among unadapted women coming to the highlands than among women who are adapted to high altitude habitats (Moran, 2000; Moore et al., 2004; Gonzales et al., 2008). Although the coastal women may have adapted to the physical stressors in the highlands over a period of time, one could argue that they had a quantitative reproductive disadvantage compared to the women who had lived in the highlands since birth (Gonzales and Tapia, 2009). Thus, the demographic maternal influence on the populations above 2500 m is too low to have a significant impact on the mitochondrial haplogroup frequencies.

Ancient DNA and the peopling of western South America

The comparison of the ancient DNA data to the continental dataset also allows some issues regarding the initial peopling of western South America to be addressed. Since the pre-Columbian regional distinctions between coast and highlands do not persist in the contemporary populations of Peru, and the ancient highland populations have a high affinity to the modern Peruvians significant changes must have taken place in the last 1000 years, causing the homogenization observed in the central Andean area (Fehren-Schmitz et al., 2010; 2011). The ancient highland and modern central Andean populations have a matrilineal genetic affinity to the Amazonian populations from eastern South America, as do the ancient coastal populations for the modern populations from the southernmost parts of South America (cf. Fig. 2; 4; 6). As mentioned before, the overall genetic variability and the genetic structure of the Native South American populations provide strong evidence that the continent was peopled by only one initial founding population (Wang et al., 2007; Lewis, Jr. and Long, 2008; Lewis, Jr., 2009). Recent studies (Lewis, Jr., 2009) also show that the patterns of genetic variability observed in South America are not consistent with a west-coast-only peopling scenario followed by a spread through the Andes into eastern South America (cf. Dillehay, 2009). Combining these discoveries with the given ancient and modern genetic data suggests a modified version of a peopling scenario previously postulated (Rothhammer et al., 2001). When the first settlers entered South America, they split up, following two or more main routes, one along the west coast, one along the north coast and then into the Amazonian Basin, and possibly an additional group along the eastern slopes of the Andes, with the latter two groups peopling the Andean highlands from the east. The settlers who chose the route along the western coast would, then, probably be the direct ancestors of the ancient coastal and modern southern South American populations. Population-biological exchange, at least matrilineal, remained marginal for the follow-
ing periods. Then, in a time frame starting possibly with the Middle Horizon and at latest with the Late Intermediate Period, populations from the Central Andean highland areas rapidly expand throughout the whole region, intermixing and demographically outnumbering the coastal populations. Of course this would not have been a simple mass migration phenomena: state-influenced migration and relocation have to be taken into account, as they are known for the Wari and Inca (Schreiber, 1992; Tung, 2007; Heggarty, 2008). The emergence of both cultures presumably also had an influence on the general migrational behaviour and the demography and genetic composition of the Central Andean populations through urbanization and changes in social complexity. Figure 2 shows that the maximum southward geographic expansion of the Inca Empire is congruent with the recent border between populations exhibiting mitochondrial distribution patterns similar to recent Peruvian populations and Chilean populations that exhibit the ancient coastal patterns. But it has to be stated that although this scenario seems plausible, it is still statistically incapable of proof on the recent data basis.

Conclusion and prospects

This study presents the potential of ancient DNA analysis for the investigation of population-dynamic processes that occurred in pre-Columbian western South America. Since the number of such studies remains small, all results and interpretations aiming at the supraregional context can only be seen as preliminary. All assumptions made in this study have been based on population affinities derived from biological distance measures and more. These methods, of course, allow no definite determination as to whether population continuities or discontinuities occurred. The data obtained has to be tested to reveal whether the differences or similarities observed could be explained by the effect over time of evolutionary forces like drift, and to reveal which demographic scenario best explains the observed values. We are currently starting a new project with Christian Anderson (Harvard University, developer of Bayesian SerialSimCoal, Anderson et al., 2005) to validate our hypotheses, comparing several complex demographic scenarios. Another recent study conducted in cooperation with Brendan O’Fallon (Felsenstein Lab, Univ. of Washington), analyzing the data presented here with Bayesian statistics, addresses the prehistoric demographic development of indigenous American populations, and it will also help to increase our understanding of the relationships of the pre-Columbian Peruvian populations (publication submitted). Although the results presented here are preliminary from the viewpoint of a statistician, the whole dataset containing genetic, ecological, and cultural data for a restricted geographic area over more than 3000 years is absolutely unique. The ongoing research will uncover more information about the reciprocal effects of cultural and environmental developments in a microevolutionary perspective.

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