Genetic Continuity After the Collapse of the Wari Empire: Mitochondrial DNA Profiles from Wari and Post-Wari Populations in the Ancient Andes

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ABSTRACT The Wari empire flourished in the central, highland Peruvian Andes from AD 600–1000, and although the events that led to its demise are unknown, archaeological evidence indicates that Wari control waned at the end of the first millennium. Here, we test the hypothesis that, despite the major shift in social and political organization at the fall of the Wari empire, the mitochondrial DNA (mtDNA) composition of populations from the Ayacucho Basin, the former imperial heartland of the empire, remained essentially unchanged. Results show that mtDNA haplogroup frequencies among the Wari and post-Wari groups differ, but the difference is not statistically significant ($\chi^2 = 5.886$, df = 3, $P = 0.1172$). This is the first study in the Andes to use haplotypic data to evaluate the observed genetic distance between two temporally distinct prehispanic populations ($F_{ST} = 0.029$) against modeled expectations of four possible evolutionary scenarios. None of these simulations allowed the rejection of continuity. In total, at both the haplogroup and haplotype levels these data do not allow us to reject the hypothesis that post-Wari individuals sampled in this study are the maternal descendants of those sampled from the Wari era site of Conchopata. However, genetic homogeneity in the mitochondrial gene pool, as seen in the late prehispanic southern Andes, may also characterize our study region. But, prior to this research, this was unknown. If our new data show mtDNA homogeneity, then this could limit the detection of female migration if, in fact, it occurred. Nonetheless, the novel mtDNA data presented here currently do not support the hypothesis that there was an influx of genetically distinct females into the former Wari heartland after the Wari collapse. Am J Phys Anthropol 000:000–000, 2009.

Analysis of mitochondrial DNA (mtDNA) has been instrumental in aiding the reconstruction of Native American prehistory by documenting genetic relationships between various precontact populations. The study of ancient DNA (aDNA) combined with archaeological data provides a unique way to reconstruct the past because assumptions about particular aspects of genetic continuity or discontinuity based on cultural proxies can be directly tested. As a complementary view to archaeological and osteological data that shows the collapse of the Wari empire in the Peruvian Andes about AD 1000, we examine if this significant cultural transformation led to alterations in the mtDNA composition of those living in the Wari imperial heartland from Wari to post-Wari times. Specifically, we inquire if natal populations living in the imperial heartland were heavily obliterated or pushed out of the region after Wari collapse, perhaps to be replaced by groups that carried substantially different mtDNA profiles. Alternatively, the subsequent inhabitants in the former Wari imperial heartland could have been descendent populations of those that lived at an earlier Wari settlement. To address this, we compare mtDNA variation exhibited by Wari and post-Wari populations. The Wari samples come from the site of Conchopata in the modern city of Ayacucho, and the post-Wari samples are from the Late Intermediate Period (post-Wari) sectors at the site of Huari, located ~10 km north of Conchopata.

We propose that, if there are distinct mtDNA compositions between the Wari (ca. AD 600–800) and post-Wari sample (AD 1100–1400), then this could have resulted from an influx of genetically distinct females into the heartland population after Wari collapse. Gene flow is the explanation for evolutionary change, when one has controlled for the evolutionary forces of mutation and genetic drift, as explained in the simulations in the Methods section. Also, a fluctuation in the gene pool in this short time span is likely not due to natural selection, as mtDNA is under weak selection (Kivisild et al., 2006). In contrast, if the mtDNA compositions of the two sample groups do not significantly differ, then this could suggest that there was no influx of nonlocal females (i.e., female population remains essentially unchanged). However, if mtDNA homogeneity of late prehispanic Andean populations, as detected in Southern Peru and Northern Chile (Lewis, In Press), extends to our study region, then it is possible that long-range population movements and/or replacements might not be detectable based on

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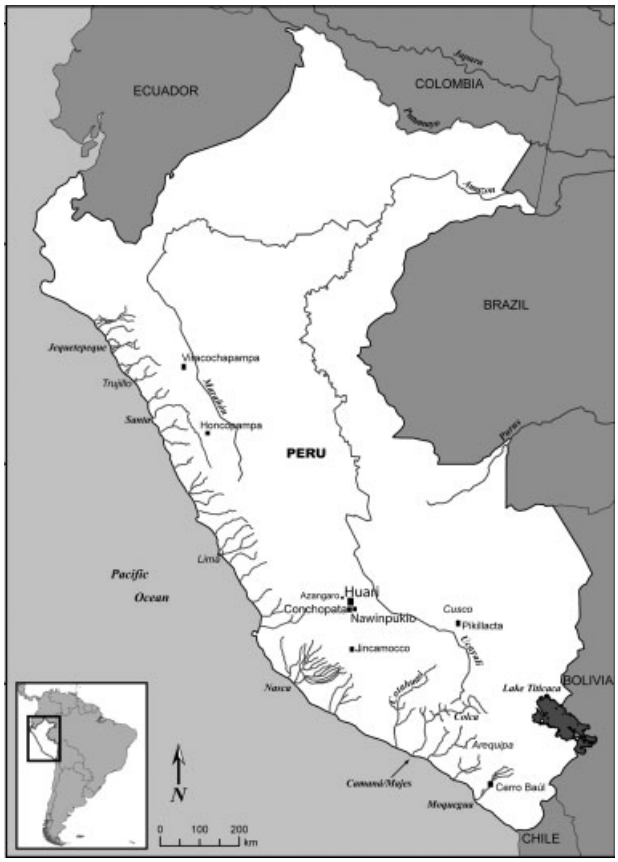

Fig. 1. Map of Peru showing the locations of Conchopata, Huari, Nawinpukio, and other sites mentioned in the text.

mtDNA analysis alone. This study does not present data that bear on the question of genetic admixture from males (i.e., as could be assessed from patterns of Y-chromosome variation).

BACKGROUND ON THE WARI EMPIRE AND ITS COLLAPSE

The Wari originated in the central highland Andes in the Ayacucho Basin of Peru about AD 600, and continued to flourish throughout the Middle Horizon, altering many aspects of social and political organization and influencing change in material culture. Wari domination is apparent in the wide distribution of Wari architecture throughout many parts of the Peruvian Andes (Feldman, 1989; Isbell, 1989; Anderson, 1991; McEwan, 1991; Moseley et al., 1991; Williams, 2001). In particular, the sites of Azangaro (Anders, 1991) and Jincamocco (Schreiber, 1992), located on the fringes of the Wari heartland, display Wari architecture, as do more distant sites such as Viracochapampa and Honcopampa in northern Peru (Isbell, 1989; Topic, 1991), Pikillacta near Cuzco (McEwan, 1991), and Cerro Baúl in southern Peru (Feldman, 1989; Moseley et al., 1991; Williams, 2001) (see Fig. 1). There are also numerous Middle Horizon sites that have Wari ceramics and textiles intrusive to the local style, illustrating the extent of Wari influence (Menzel, 1964, 1968; Stone-Miller and McEwan, 1990; Schreiber, 1992; Cook, 1994; Cardona Rosas, 2002; Cook and Glowacki, 2003; Owen, 2007; Tung, 2007a).

Wari's widespread influence and control in parts of the Andes may have partly succeeded through religious indoctrination (Menzel, 1964), but Wari military campaigns, or the threat thereof, appear to have been a particularly successful strategy for expansion (Lumbreras, 1974; Feldman, 1989). Evidence for this is seen on state-produced Wari ceramics that depict warriors in uniforms carrying shields and weapons (Ochatoma and Cabrera, 2002). Moreover, analysis of cranial fractures demonstrates that adults from the site of Conchopata and other Wari affiliated sites in the Majes valley of southern Peru frequently engaged in violent acts, as ~30% show head trauma (Tung, 2007b). In short, the Wari empire exerted much influence throughout the Andes, affecting architectural design, material culture, ritual practices (Cook, 2001; Tung, 2008), road systems and trade networks (Schreiber, 1991), food production (Goldstein et al., In press; Tung, 2007a), and morbidity profiles of subject and ruling groups (Drusini, 2001; Kellner, 2002; Tung, 2007b). Throughout this process, diverse groups of people were brought under the umbrella of Wari authority, but current evidence suggests that foreigners were not brought to the Wari heartland as immigrants. Although iconographic and strontium isotope evidence suggests that Wari military agents traveled to distant hinterland regions, current strontium isotope ratios from Conchopata skeletons suggest that no immigrant groups settled at the site (Tung and Knudson, 2008; Tung et al., 2008). Thus, it is unlikely that "foreigners" at the Wari site of Conchopata are included in our Wari era samples.

For reasons yet unknown, the Wari empire came to an end approximately four centuries after its inception. The subsequent era (Late Intermediate Period) has been generally described as a time of political and social instability characterized by a political power void within which various polities jockeyed for authority (Willey, 1991; Arkush, 2005). In the Wari imperial heartland in particular, there was a concomitant increase in violence, as evidenced by antemortem cranial trauma frequencies of more than 70% (Tung, 2009). There was also a major increase in the number of individuals with cranial modification from Wari to post-Wari times; this was a practice whereby the shape of an infant's head was altered by placing small boards and cordage around the cranium. This kind of body modification was a visible marker of ethnic identity in the prehispanic Andes (Torres-Rouff, 2002; Blom, 2005), and the increase of this practice in post-Wari times suggests that either new corporeal expressions of identity were created or a distinct population moved into the former Wari imperial heartland. Given changes in material culture, incidences of violence, and physical expressions of identity, we investigate whether there was a significant shift in mtDNA variation from one time period to the next. If mtDNA haplogroup and haplotype frequencies significantly differ between the Wari and post-Wari samples, then this may suggest that outsider females (and perhaps their kin and other community members) migrated to the former Wari heartland after the collapse of this once dominant empire. In contrast, mtDNA continuity between the temporally distinct populations would suggest that the post-Wari peoples were descendents of the Wari who continued to reside and reproduce in what was once the core of Wari imperial territories.

Given the potential confusion between the terms "Wari" and "Huari", we follow Isbell's (2004) nomenclature and use "Wari" to refer to the culture and cultural

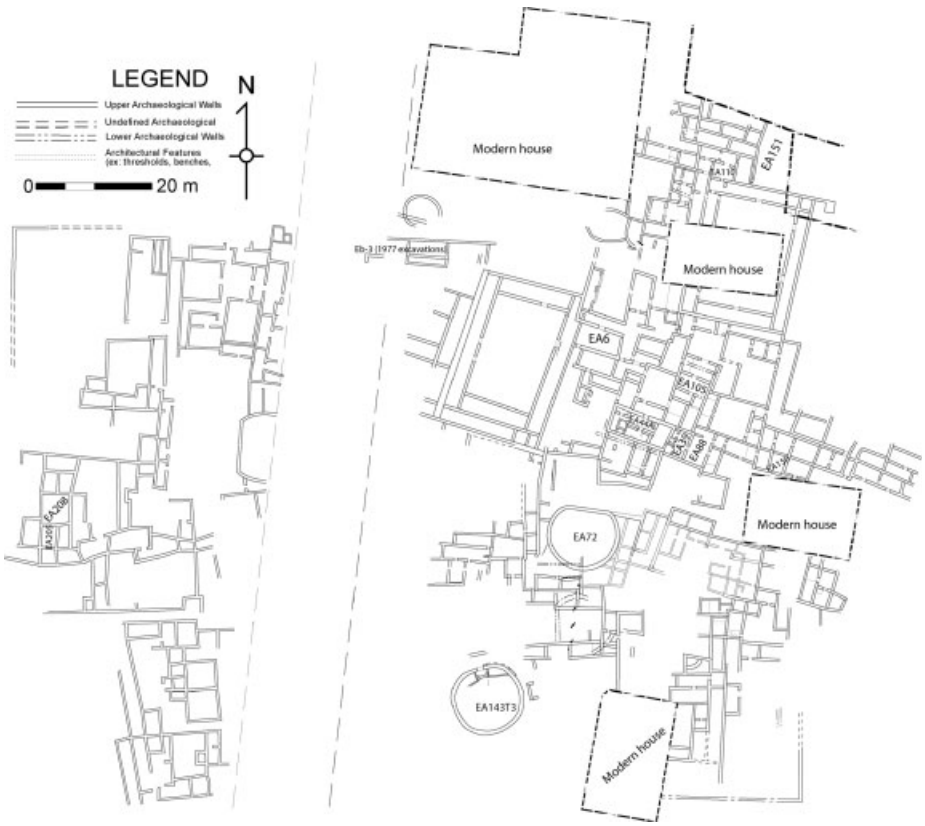

Fig. 2. Map of Conchopata showing the locations of architectural spaces from which osteological samples were taken. (Based on map created by Juan Carlos Blacker).

material, and "Huari" to refer specifically to the capital site. The site of Huari was used during both the Middle Horizon (Wari era, AD 600–1000) and the Late Intermediate Period (post-Wari, AD 1000–1450). Huari was the capital of the Wari empire, and in the subsequent post-Wari era, parts of the site, such as Monqachayoq and Vegachayoq Moqo, were reused as mortuary areas. Radiocarbon dates obtained directly from human bones at these two sectors at Huari confirm that they indeed date to the Late Intermediate Period (Tung, 2009).

BACKGROUND ON ANCIENT DNA STUDIES IN THE ANDES

Our approach to addressing prehistory in the central Peruvian Andes with mtDNA evidence is similar to genetic studies previously conducted on prehistoric populations in the southern Peruvian Andes, for example, studies of the Tiwanaku-affiliated site of Chen Chen (Lewis et al., 2007) and various Northern Chilean sites dating from the Late Archaic to the Late Horizon (Moraga et al., 2005). In the latter case, Moraga et al. (2005) observed a major change in ancient mtDNA haplogroup frequencies in Northern Chilean valleys over a 3,900 year period. They suggested that this change could have resulted from the migration (gene flow) of individuals from the Andean highlands, a scenario consistent with observed craniofacial morphological change (Rothhammer and Santoro, 2001). However, Lewis et al. (2007) found that the frequencies reported by Moraga et al. (2005) from the Late Archaic to Late Intermediate Period did not differ significantly (Fisher's exact test, $P = 0.658$), and thus, were not indicative of any significant gene flow. Moreover, Lewis and colleague's (2007) comparison of mtDNA variation between the Chen Chen skeletal samples and 58 ancient and extant South American populations revealed that haplogroup homogeneity has dominated the central Andean region of southern Peru and northern Chile since at least the time before the Middle Horizon when the Wari empire and Tiwanaku state flourished. Whereas statistically different patterns of mtDNA variation can be detected across time and space among Andean populations, Lewis et al. (In Press) have recently demonstrated that genetic drift, and not population replacement or gene flow, is the simplest explanation for these interpopulational discrepancies. For our case study, it is possible that mtDNA patterns observed in populations of the central Peruvian Andes may be similar to those observed in the Tiwanku polity during the Middle Horizon (Lewis et al., 2007). If there was wide-spread homogeneity during this time, detecting genetic discontinuity based solely on variation in the mitochondrial genome may be difficult over the time period considered in this study, regardless of the magnitude of any possible population movement witnessed at the local level. However, this is unknown and remains to be tested in our study. To further evaluate this possibility, our study includes haplotypic data which provides a higher power of discrimination over simply screening the samples for the defining markers of the four most frequent Native American mtDNA haplogroups (A, B, C, or D), which provides only three degrees of freedom in the chi-square test, as highlighted by Lewis et al. (in press). Here, for the first time, haplo-

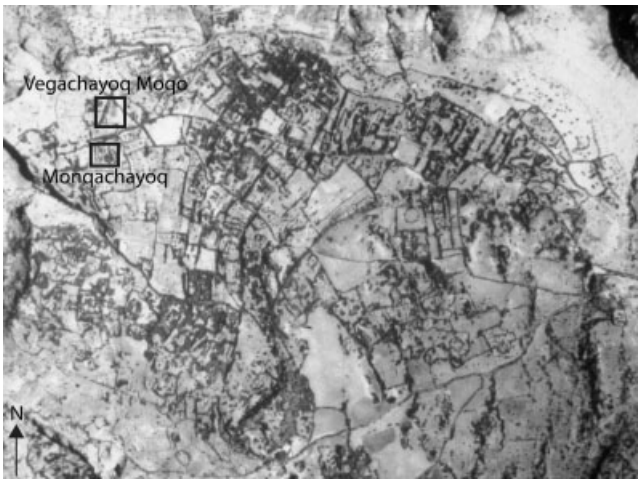

Fig. 3. Aerial photo of Huari. The sectors of Monqachayoq and Vegachayoq Moqo are outlined. Photo courtesy of Servicio Aerofotográfico Nacional de Peru.

typic data are used to evaluate observed genetic distances between two temporally distinct Andean populations against the modeled expectations of four possible evolutionary scenarios. With our sampling strategy and focus on mtDNA, this haplotypic approach stands as the best possible means for evaluating population dynamics in our study area given the samples at hand.

MATERIALS AND METHODS

The samples

Seventy-four osteological samples were collected from 69 archaeological individuals for genetic analysis. To test the reliability of the techniques, two or three bone and tooth samples from the same individual were taken by Tung, but not reported to Kemp, who conducted the DNA extraction and mitochondrial DNA haplogroup and haplotype determination, until all mtDNA results were available. The samples come from three archaeological sites in the Ayacucho Basin: 1) Nawinpukio (Wari era samples, $n = 6$), 2) Conchopata (pre-Wari samples, $n = 2$ and Wari era samples, $n = 47$), and 3) Huari (post-Wari samples from two sectors at the site known as Monqachayoq ($n = 15$) and Vegachayoq Moqo ($n = 4$) (see Fig. 3). Tung excavated the Conchopata burials as part of the Conchopata Archaeology Project, directed by William Isbell and Anita Cook, and was thus able to select osteological samples that were the most intact and had the least disturbed associated contexts (many of the burials had been looted) (see Fig. 2). Nawinpukio burials were excavated by Martha Cabrera in the mid-1990s. These burials were placed in simple circular pits without any stone-lining. Enrique Bragayrac excavated the post-Wari Vegachayoq Moqo remains in the 1980s; those burials derive from the base of a stone wall and in stone-lined niches in the Vegachayoq Moqo sector. Francisco Solano excavated the post-Wari Monqachayoq human remains in 1977–78. The Monqachayoq human remains come from Gallery 3, an underground, stone-lined mortuary gallery that measured 12 m in length and averaged 2 m in height (see Fig. 4) (Solano Ramos and Guerrero Anaya, 1981). These large, stone-lined galleries that were constructed underground created a cool microclimate, which we hypothesized would lead to better DNA preservation. In contrast, the Conchopata burials were interred in shallow cylindrical tombs, only some of which were stone-lined, whereas other bodies were placed in cavities cut into the bedrock. We hypothesized that the post-Wari Huari remains would provide a higher rate of successful mtDNA extraction relative to Nawinpukio and Conchopata, due in large part to their distinct burial environments.

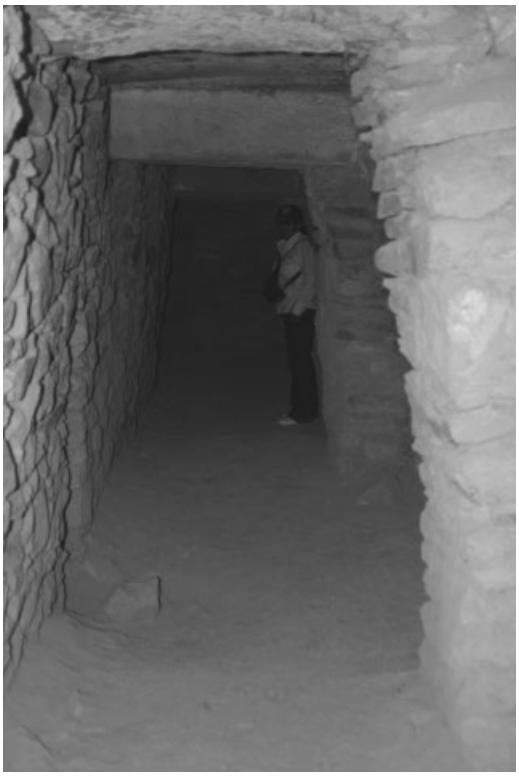

Fig. 4. Underground, stone-lined burial gallery at the Monqachayoq sector at the site of Huari.

In the years between excavation and analysis, Conchopata remains were stored in acid-free paper and aluminum foil and stored in cardboard boxes. The Nawinpukio remains were stored in plastic bags, and the Monqachayoq and Vegachayoq Moqo bones were wrapped in newspaper and stored in cardboard boxes. None of the human remains were washed with water. In cases where dirt adhered to the bones or teeth, the dirt was brushed off with a synthetic fiber brush or picked away with a wooden dowel.

Molecular methods

All of the pre-PCR methods described below were conducted in the newly established ancient DNA laboratory in the Center for Human Genetics Research at Vanderbilt University, wherein the equipment and reagents are dedicated for processing degraded specimens alone. This pre-PCR lab is physically separated from the labs where modern human DNA studies are conducted and from that of the post-PCR lab. Access to this laboratory is restricted to ancient DNA researchers and precautions to minimize contamination were always practiced. These precautions included: 1) wearing clean lab coats, hair nets, and disposable latex gloves, 2) regularly decontaminating the workspaces with a 0.6% sodium hypochlorite

solution, and 3) once having worked in the post-PCR lab, only returning to the ancient DNA lab after taking a shower and changing clothes.

DNA extraction

Before attempting DNA extraction from the tooth samples (Table 1), the crowns and roots were carefully separated by sawing through the tooth at the cervix. A new saw blade was used on each individual sample to avoid cross contamination. From the remaining tooth root and bone samples, ~0.1–0.6 g of material was removed from the whole for DNA extraction. Because aDNA often occurs in low copy number and is highly degraded (Pääbo, 1990; Lindahl, 1993), aDNA extractions are highly susceptible to contamination from modern sources. Therefore, the samples were submerged in 6% sodium hypochlorite (full strength Clorox Bleach) for 15 min to remove any surface contamination (Kemp and Smith, 2005). The bleach was poured off and the samples rinsed with DNA-free ddH_2O (Gibco) 1–3 times to remove any remaining bleach. The samples were transferred to a 15 mL conical tubes and demineralized by gentle rocking in 2-mL molecular grade 0.5 M EDTA, pH 8.0 (Gibco), for >48 h at room temperature. Three milligrams of proteinase K were added to the sample, followed by incubation at 65°C for at least 3 h. DNA was then extracted from 1 ml of the EDTA using the MagNA Pure LC DNA Isolation Kit (large volume). The MagNA Pure system is an automated one that uses magnetic silica beads to bind DNA and carry it through the extraction process. The settings and preparation of the MagNA Pure system followed the instructions of the manufacturer. Extraction blanks, consisting of EDTA and proteinase K, accompanied each set of extractions to allow for the detection of contamination originating during the extraction.

Haplogroup determination and sequencing

The samples were screened for the defining mutations of Native American mitochondrial haplogroups A, B, C, and D (Schurr et al., 1990; Forster et al., 1996). Samples were not screened for the markers definitive of haplogroup X, as this mitochondrial type has not been described in any South American populations (Dornelles et al., 2005) and all of the sample in this study were determined to belong to either haplogroup A, B, C, or D (see Results). Screening of these molecular markers was conducted according to Kemp et al. (2007). Those samples that failed to produce amplicons during this initial screening process were tested for failure due to the presence of coextracted PCR inhibitors, as described by Kemp et al. (2006). For initial inhibition tests, DNA re-extracted from previously described Aztec populations (Kemp et al., 2005; De la Cruz et al., 2008) as just described were used as "positive aDNA controls." In later tests for PCR inhibition, DNA extracted from Conchopata and Huari samples were used as "positive aDNA controls."

Nucleotide positions (nps) 16011–16382 of the first hypervariable region (HVRI) of the mitochondrial genome were sequenced in those samples that were determined to belong to haplogroup A, B, C, or D. Sequencing was not attempted in those cases when a sample failed to produce amplicons during the screening of the haplogroup defining mutations. HVRI was amplified and sequenced in four, small (<175 bp) overlapping fragments using primers sets "D-Loop 1, 2, 3, and 4" and the PCR conditions described by Kemp et al. (2007). A fifth pair of primers was designed and optimized during this study to complete sequence overlap in samples that exhibit a cytosine (C) at nucleotide position (np) 16189. This mutation results in a poly-C stretch in the amplicon targeted by the primer set "D-Loop 2" that currently cannot be sequenced through. In the case that a sample exhibits a transition at np 16189, with no additional mutations to "anchor" the region, the reverse sequence of the "D-Loop 2" amplicon fails to overlap with the "D-Loop 1" sequence. To fill in this gap, the following primers were used to amplify and sequence a fifth overlapping amplicon: 16001F (ATTCTAATTTAAACTATTCTCTG) and 16181R (TTGATGTGGATTGGGTTTTT). For this amplicon, 30 μL reactions contained: 0.32 mM dNTPs, 1× PCR Buffer, 1.5 mM $MgCl_2$, 2.4 mM primers, 0.3 U of Platinum *Taq* (Invitrogen), and 3.0 μL of DNA template. This reaction was subjected to 60 cycles of PCR, as follows: 3 min denaturing at 94°C, followed by 15 second holds at 94°C, 50°C, and at 72°C, followed by a final 3 min extension period at 72°C. Sequence preparation followed that described by Kemp et al. (2007). All sequencing products were submitted for direct sequencing to the DNA Sequencing Facility at Vanderbilt University. Sequencing was performed in both directions and sequences were read off both strands. Sequences that represented contamination arising from the author that performed the molecular work (B.M.K.) were discarded. In addition, heteroplasmic sequences that could not be confirmed were also discarded, as these most likely arose from either mixed sequences (i.e., a mixture of contamination and authentic aDNA) or post mortem damage to the template DNA (e.g., deamination).

Confirmation of molecular results

A second DNA extraction was performed, as described above, on all of the samples that yielded positive haplogroup and sequencing results. These extractions were screened for the molecular markers and sequenced as described earlier.

Haplogroup and haplotype analysis

The haplogroup frequencies exhibited by the Conchopata and Huari populations were compared using a chi square test conducted in SPSS. An alpha value of 0.05 was selected as the level of significance.

As a measure of genetic distance between the two populations, F_{ST} was calculated in Arlequin (v 2.000) (Schneider et al., 2000). Samples Concho 08, Concho 34, and Huari-MQ-02 (Table 2) were not included in this estimation of F_{ST} due to large amounts of missing data in their respective sequences. To assess whether the observed F_{ST} value was not larger than expected due simply to the evolutionary forces of genetic drift and mutation, Serial SimCoal (Excoffier et al., 2000; Anderson et al., 2005) was used to model evolution over the 800 years that separated the Conchopata and Huari populations. As 800 years represents the maximum time separating the two populations, the simulations are conservative in allowing for a maximum amount of evolutionary change. A generation time of 20 years was assumed, amounting to ~27 generations separating the two groups. This input parameter again results in a con-

TABLE 1. List of the osteological samples and their archaeological contexts and the mtDNA results when available

Site	Time period	Lab ID	Architect. space	Locus	Excavation burial code	Osteology Lab Code	Bone/tooth	mtDNA Hg
Conchapata	MH	950.01	44A	950		950.01	Molar 3	?
Conchapata	MH	2095.02	105	2095		2095.02B	Rib	?
Conchapata	MH	2112.01	110	2112		2112.02	L Md molar 1	?
Conchapata	MH	2095.01B	105	2095		2095.01B	Rib	?
Conchapata	MH	2095.01T	105	2095		2095.01T	Tooth	?
Conchapata	MH	2095.04B	105	2095		2095.04B	Rib	?
Conchapata	MH	2095.04T	105	2095		2095.04T	Tooth	?
Conchapata	MH	2095.06B	105	2095		2095.06B	Rib	?
Conchapata	MH	2095.06T	105	2095		2095.06T	Tooth	?
Conchapata	EIP	Eb-3 Rib		Tomb 5		5.01	Rib	?
Conchapata	EIP	Eb-3 Tooth		Tomb 5		5.03	R Mx premolar 4	?
Conchapata	MH	1993.01	110	1993		1993.01	L Md molar 1	B
Conchapata	MH	Concho 01	150	2981	94.04	2981.04	metacarpal	?
Conchapata	MH	Concho 02				Ochatoma Ind. 1	L metacarpal	D
Conchapata	MH	Concho 03	44A	950		950.02	R humerus frag	?
Conchapata	MH	Concho 04	150	2981	94.02	2981.02	R ulna	?
Conchapata	MH	Concho 05	205	3521	104.01	3521.01.64	L fibula	?
Conchapata	MH	Concho 06	205	3521	107.03	3521.03.66B	R hand phalanx	?
Conchapata	MH	Concho 07	205	3554	106	3554.01.44	R fibula	?
Conchapata	MH	Concho 08	205	3521	105.02	3521.02.55	L rib	C
Conchapata	MH	Concho 09				Ochatoma Ind. 1	L metatarsal	D
Conchapata	MH	Concho 10	150	2981	94.01	2981.01	LB frag	?
Conchapata	MH	Concho 11	143T3	2985	21	2985.21.01	Cranial frag	?
Conchapata	MH	Concho 12	187	3335		3335.155	R Mx canine	B
Conchapata	MH	Concho 13	39A	1818		1818.01	Cran frag	?
Conchapata	MH	Concho 14	39A	1818		1818.02	Md molar 1	?
Conchapata	MH	Concho 15	Airport tower			Airport tower	Md molar 3	?
Conchapata	MH	Concho 16	39A	1818		1818.03	Cran frag	?
Conchapata	MH	Concho 17	39A	1818		1818.04	Md molar 1	?
Conchapata	MH	Concho 18	72			Ochatoma trophy D	Md molar 1	?
Conchapata	MH	Concho 19	72			Ochatoma trophy C	Md molar 2	?
Conchapata	MH	Concho 20	205	3521	107.03	3521.03.18T	R premolar 3	?
Conchapata	MH	Concho 21	208	3547	108.03	3547.03.05	L incisor 2	B
Conchapata	MH	Concho 22	88	3032	54.03	3032.03.06	R canine	C
Conchapata	MH	Concho 23	208	3547	108.01	3547.01.13	L Mx molar 2	A
Conchapata	MH	Concho 24	208	3577	108.05	3577.05.01	RM1	?
Conchapata	MH	Concho 25	20	1371		1371.01.05	L Md canine	B
Conchapata	MH	Concho 26	151	2858	51.01	2858.01	R Mx premolar 4	A
Conchapata	MH	Concho 27				Ochatoma Ind. 1	Md molar 3	D
Conchapata	MH	Concho 28	110	2112.1		2112.01	L Md molar	?
Conchapata	MH	Concho 29	39A	1728		1728.01	Md LP3	A
Conchapata	MH	Concho 30	39D	2026		2026.01	Md molar	B
Conchapata	MH	Concho 31	110	1993		1993.02	R Md molar 1	A
Conchapata	MH	Concho 32	Surface	1799		1799.01	Md molar	?
Conchapata	MH	Concho 33	?	2200		2200.01	Mx molar	?
Conchapata	MH	Concho 34	39D	2045		2045.01		B
Conchapata	MH	Concho 35	6	2004		2004.01		?
Conchapata	MH	Concho 36	?	2400		2400.54	Md molar 3	B
Conchapata	MH	Concho 37	105	2095		2095.03B	Rib	?
Huari-MQ	LIP	Huari-MQ-01				AY	Radius frag	?
Huari-MQ	LIP	Huari-MQ-02			1 II 2-5	1 II 2-5	Rib frag	C
Huari-MQ	LIP	Huari-MQ-03			50	50		C
Huari-MQ	LIP	Huari-MQ-04				Q		A
Huari-MQ	LIP	Huari-MQ-05			30	30		C
Huari-MQ	LIP	Huari-MQ-06			8	8		C
Huari-MQ	LIP	Huari-MQ-07			2	2	Mx molar 2	A
Huari-MQ	LIP	Huari-MQ-08			15	15	Mx molar 2	B
Huari-MQ	LIP	Huari-MQ-09			23	23	Mx molar 2	C
Huari-MQ	LIP	Huari-MQ-10			51	51	Mx molar 1	C
Huari-MQ	LIP	Huari-MQ-11			69	69	Mx molar 3	C
Huari-MQ	LIP	Huari-MQ-12			70	70	Mx molar 1	C
Huari-MQ	LIP	Huari-MQ-13				M	Mx molar 3	D
Huari-MQ	LIP	Huari-MQ-14				P	L Mx molar 2	A
Huari-MQ	LIP	Huari-MQ-15				S	R Mx molar 2	B
Huari-VM	LIP	Huari-VM-01			66	66		C
Huari-VM	LIP	Huari-VM-02			4	4		B
Huari-VM	LIP	Huari-VM-03			58	58		C
Huari-VM	LIP	Huari-VM-04				1001	R Mx molar 2	B
Nawinpukio	MH	1	Fosa 2		F2.02.01	F2.02.01	R Md premolar 3	?
Nawinpukio	MH	2	Fosa 2		F2.02.02	F1.01	Md R molar	?

TABLE 1. (Continued)

Site	Time period	Lab ID	Architect. space	Locus	Excavation burial code	Osteology lab code	Bone/tooth	mtDNA Hg
Nawinpukio	MH	3	Fosa 2		F2.01.01	F2.01.01	Cran frag	?
Nawinpukio	MH	4	Fosa 2		F2.05	F2.05	Cran frag	?
Nawinpukio	MH	Naw-1	Fosa 1		F1.01	F1.01	Molar	?
Nawinpukio	MH	Naw-2	Fosa 2		F2.04	F2.04	Cran frag	?

servative approach to modeling population evolution, as generation time in contemporary humans has been estimated to be $\geq$30 years (Siguroardottir et al., 2000). Input parameters that varied in the simulations were effective female population size (N_{ef}) and mutation rate. In the case of N_{ef}, input values of 100 ("Small") and 1,000 ("Large") were used to explore the expectation of F_{ST} over an order of magnitude difference in female population sizes. Importantly, this range of effective population sizes nearly encompasses that estimated for recent populations by Hey (2005) to that estimated more recently by Mulligan et al. (2008) for the founding Native American population. The rate of molecular evolution was also varied in the simulations to consider rates derived from pedigree studies ("Fast") and phylogenies ("Slow") (see discussion by Kemp et al., 2007). Importantly, consideration of the range of evolution rates helps control for the recent debate about decaying rates of observable evolution (Ho et al., 2005; Ho and Larson, 2006). The "Fast" rate input was 0.0035 mutations per generation over the 373 bp region, which was calculated from a pedigree-estimated rate of 47.5%/bp/myr (Howell et al., 2003) and the assumption of 20 year generations. This rapid rate of evolution estimated from pedigrees has been shown to hold over the past 10,300 years (Kemp et al., 2007), and thus is likely a good estimation for the time period under consideration in the present study. The "Slow" rate input was 0.0011 mutations per generation over the 373 bp region, which was calculated from the average of phylogenetic-estimated rates, ~15%/bp/myr (Bonatto and Salzano, 1997; Stone and Stoneking, 1998), and the assumption of 20 year generations. In total, there were four starting conditions for the simulations: 1) Small, Fast, 2) Small, Slow, 3) Large, Fast, and 4) Large, Slow (See Appendix for the "Small, Fast" Serial SimCoal input file). Each of these respective simulations was run 10,000 times. An alpha value of 0.05 was chosen as the level for significant departure from expected F_{ST} values.

RESULTS

The genetic profiles

None of the six Nawinpukio samples yielded DNA. Sixteen of the 49 Conchopata specimens (33%), representing 14 individuals, and 18 of 19 Huari individuals (95%) contained analyzable mtDNA. There was a significant difference in the long-term preservation of DNA in the Conchapata and Huari samples ($\chi^2 = 21.1$, $df = 1$, $P < 0.0001$). All samples that contained aDNA could be assigned to one of the Native American haplogroups (Table 1). Three individuals for whom bone-tooth pairs were tested yielded no results (2,095.01, 2,095.04, and 2,095.06), but one individual for whom two bones (Concho 02, 09) and one tooth (Concho 27) were tested all yielded haplogroup D, confirming the reliability of these methods (Kemp was unaware that these three samples were from the same skeleton). Moreover, the sequence data retrieved from Concho 02, 09, and 27 (all from the same individual) were identical (see note in Table 2). We also note that the absence of positive results was not due to the coextraction of PCR inhibitors; rather, using the methods described earlier, those samples that yielded no results appear to be void of analyzable mtDNA.

The Conchopata population ($n = 14$) exhibited 28.6% haplogroup A, 50% haplogroup B, 14.3% haplogroup C, and 7.1% haplogroup D (see Fig. 5). The post-Wari Huari population ($n = 18$) exhibited 16.7% haplogroup A, 22.2% haplogroup B, 55.5% haplogroup C, and 5.6% haplogroup D (see Fig. 5). The haplogroup frequencies between the two sample populations are statistically indistinguishable ($\chi^2 = 5.886$, $df = 3$, $P = 0.1172$).

HVRI sequences were obtained from 10 of the 14 Conchopata individuals and 18 of the 19 Huari samples (Table 2). Missing sequence data in Table 2 stem from failed amplification in one or more amplicons; sequences determined in one extraction, but not confirmed in the second, are not reported. Two haplotypes are shared across the populations. The first, shared by Concho 02 and Huari-MQ-13, is a haplogroup D haplotype exhibiting transitions at nps 16223, 16325, and 16362, relative to the Cambridge reference sequence (CRS) (Anderson et al., 1981; Andrews et al., 1999). This sequence represents the founder type of subhaplogroup D1 in the Americas (Tamm et al., 2007). Concho 22, Huari-MQ-02, and Huari-MQ-09 share the second type, which belongs to haplogroup C; it exhibits transitions at nps 16223, 16298, 16325, and 16327. This sequence represents the founder of any one of the three subhaplogroup C1s carried to the Americas (Tamm et al., 2007).

All results reported here were confirmed from two extractions of each sample and rigorous decontamination of the samples was conducted before extraction. All of HVRI sequences are consistent with the samples respective haplogroup assignments, in that they exhibit haplogroup specific motifs (Tamm et al., 2007). Moreover, the HVRI sequences were compiled from overlapping fragments from multiple amplifications that strongly suggest that the results are authentic and unaffected by DNA damage or contamination.

The estimated F_{ST} between the two populations is 0.029. This observation falls within the 95% confidence interval of expected values for all four simulations performed in Serial SimCoal (see Fig. 6)

DISCUSSION

The much higher success rate of DNA extraction for the Late Intermediate Period Huari samples was likely related to their burial environments. Nawinpukio and Conchopata burials were in shallow tombs and in direct contact with soil, factors that likely contributed to the low success rate in recovering ancient mtDNA (0 and 33%, respectively). The post-Wari burials at Huari, in contrast, were primarily surrounded by stone. In particular, the Monqachayoq skeletons were buried in an underground, stone-lined gallery (see Fig. 4) that created

TABLE 2. First hypervariable region (HVRI) sequences exhibited by individuals sampled from the sites of Conchopata and Huari

	Haplogroup	16017	16093	16104	16111	16114	16124	16129	16156	16157	16179	16182	16183	16189	16192	16217	16222	16223	16258	16259.1	16260	16261	16270	16274	16286	16289	16290	16291	16298	16316	16319	16325	16327	16356	16357	16362	Sequence read
CRS	H	T	T	C	C	C	T	G	G	T	C	A	A	T	C	T	C	C	A	-	C	C	C	G	C	A	C	C	T	A	G	T	C	T	T	T	N/A
Concho23	A	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	T	.	-	.	.	.	A	.	.	T	.	.	.	A	C	.	.	.		16011–16382
Concho26	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	C	16011–16382
Concho12	B	.	.	.	.	.	.	.	.	.	.	.	C	C	.	C	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	16011–16382
Concho25	B	.	.	.	.	.	.	.	.	.	.	C	C	C	.	C	.	.	G	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	16011–16382
Concho30	B	.	.	.	.	.	.	.	.	.	.	C	C	C	.	C	.	.	.	-	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	16011–16382
Concho34	B	?	?	?	?	?	?	?	?	?	?	?	?	?	?	C	.	.	.	-	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	16210–16382
Concho36	B	.	.	.	.	T	.	.	.	.	T	C	C	C	.	C	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16011–16382
Concho22	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.	.	.	.	.	.	.	C	.	.	C	T	?	?	?	16011–16330
Concho08	C	?	?	?	?	?	?	?	?	?	?	?	?	?	?	.	.	.	.	-	.	.	.	.	T	.	.	.	C	.	.	C	T	.	.	.	16210–16382
Concho02	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	16011–16382
Huari-MQ-07	A	.	.	T	T	.	.	.	?	?	?	?	?	?	?	?	?	?	.	-	.	.	.	.	.	.	T	T	.	.	A	.	.	.	.	C	16011–16131 16250–16382
Huari-MQ-04	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	C	16011–16382
Huari-MQ-14	A	.	.	.	T	.	.	A	.	.	.	.	.	.	.	.	.	T	.	-	T	.	.	.	.	.	T	.	.	.	A	.	.	.	.	C	16011–16382
Huari-MQ-08, 15	B	.	.	.	.	.	.	.	.	.	.	C	C	C	.	C	.	.	.	-	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16011–16382
Huari-VM-02	B	C	.	.	.	.	.	.	.	.	.	C	C	C	.	C	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	16011–16382
Huari-VM-04	B	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	-	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	16011–16382
Huari-MQ-02	C	?	?	?	?	?	?	?	?	?	?	?	?	?	?	.	.	T	.	-	.	.	.	.	.	.	.	.	C	.	.	C	T	.	.	.	16210–16382
Huari-MQ-03, 05, 10, VM-01	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.	.	.	.	.	.	.	.	.	.	C	T	.	.	.	16011–16382
Huari-MQ-06	C	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	T	T	.	-	.	.	.	.	.	.	.	.	C	G	.	C	T	.	.	.	16011–16382
Huari-MQ-09	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.	.	.	.	.	.	.	C	.	.	C	T	.	.	.	16011–16382
Huari-MQ-11	C	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	T	.	-	.	.	.	.	.	.	.	.	C	.	.	C	T	.	.	.	16011–16382
Huari-MQ-12	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	T	.	.	.	.	.	.	.	C	.	.	C	T	.	.	.	16011–16382
Huari-VM-03	C	.	.	.	.	.	.	.	A	Y	.	.	.	.	.	.	.	T	.	-	.	.	.	.	.	.	.	.	C	.	.	C	T	.	.	.	16011–16382
Huari-MQ-13	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	16011–16382

Total stretch of each sequence is indicated on the right.
?, missing sequence data; y, C/T heteroplasmy detected in the sample.
This observation was confirmed in sequences generated from multiple extractions from the sample. Concho 09 and 27 (Table 1) represent a bone and tooth, respectively, sampled from the same individual represented by Concho 02 in this table. Sequences from these samples, determined through only one extraction on each, match the haplotype exhibited by Concho 02, Concho 09 was sequenced from nps 16,011–16,032, and exhibited: 162,23T, 163,25C, and 163,62C. Sequence data from Concho 27 was only retrievable from nps 16,250–16,382 and exhibited the 16,362C transition.

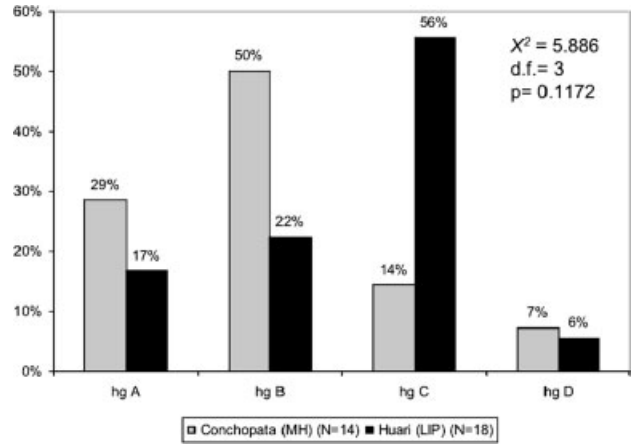

Fig. 5. Haplogroup frequencies for Conchopata and Huari.

a cool microclimate, apparently ideal for long-term DNA preservation (95% recovery of mtDNA).

The Conchopata and post-Wari Huari burial samples cannot be statistically differentiated at the haplogroup level and, thus, the former cannot be excluded as being directly ancestral to the latter on the direct maternal line. However, this conclusion is contingent on the assumption that our samples are representative of the once-living population against which the deviations are being measured. Notwithstanding the limited sample size, another assumption holds that immigrants to the former Wari heartland carried a significantly different mtDNA profile, which thereby would have provided the means for genetic analysis to detect the population influx. In other words, if a population replacement occurred in the Ayacucho Basin in the post-Wari era, but there exists spatial continuity in mtDNA variation throughout the Andes, as suggested by research in the southern Andes (Lewis, (In Press); Lewis et al., 2007), a significant chi square value may not be obtainable without large and representative samples sizes. Although we could not sample additional Middle Horizon populations to examine the haplogroup homogeneity more generally, we could compare our Wari samples to others of similar antiquity from elsewhere in the Andes. These previously published samples include the population that resided at the Tiwanaku-affiliated site of Chen Chen around AD 785–1000 (Lewis et al., 2007), and the ~1000-year-old Middle Sicán burials from the northern Peruvian coast (Shimada et al., 2004). In both comparisons, the populations are statistically indistinguishable from the Conchopata samples (Conchopata vs. Chen Chen $\chi^2 = 0.69$, $df = 3$, $P = 0.875$, Conchopata vs. Sicán $\chi^2 = 4.0$, $df = 3$, $P = 0.26$). It is evident from these tests that, at the haplogroup level, the major spatial genetic continuity existed across Peru during the latter part of the Middle

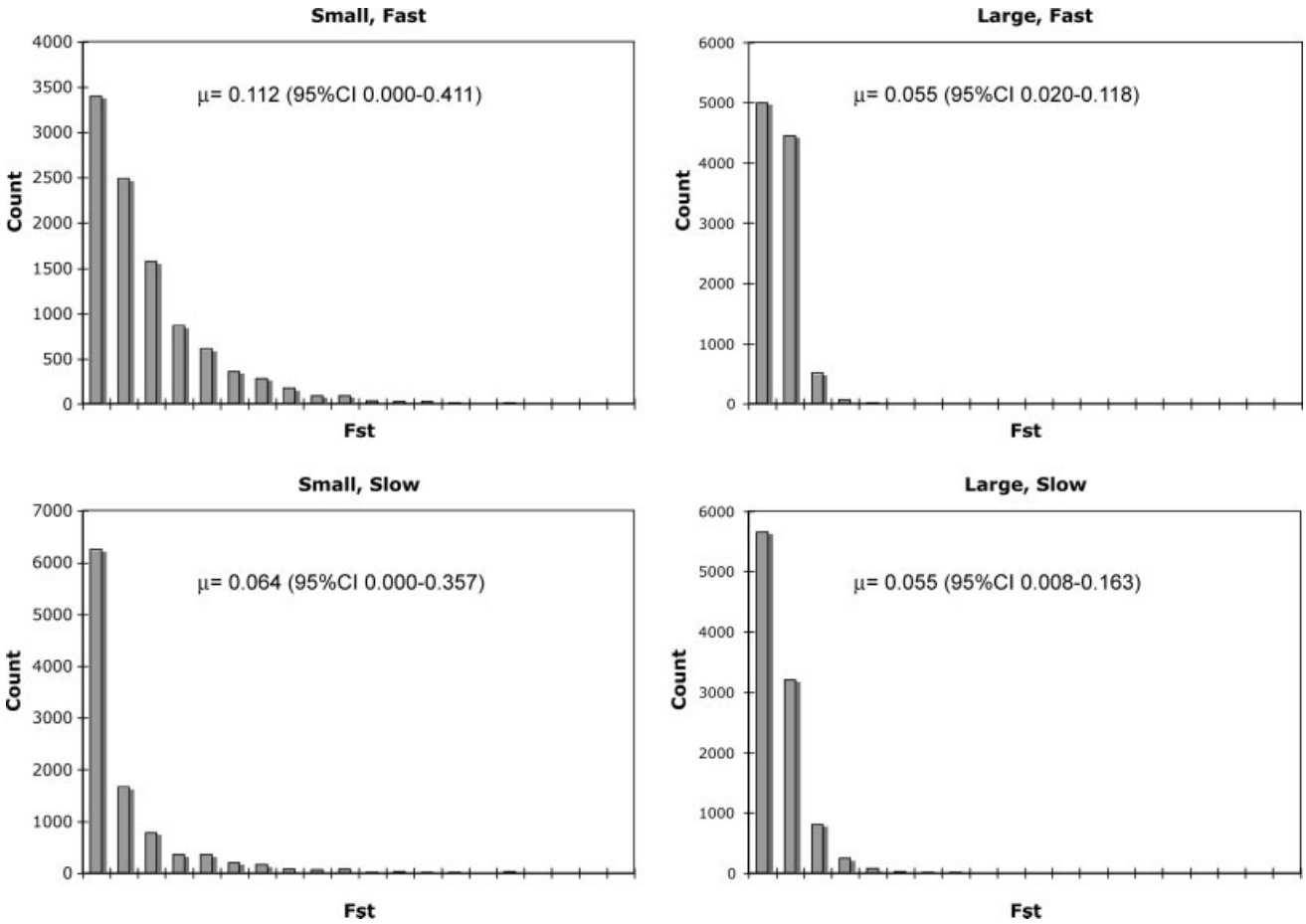

Fig. 6. Distribution of expected F_{ST} values from the four Serial SimCoal simulations. The observed F_{ST} between Conchopata and Huari was 0.029.

Horizon. In this case, our conclusion that the post-Wari individuals buried at Huari are the descendants of those buried at Conchopata will require further testing with larger samples sizes and, if possible, by screening genetic markers in the nuclear genome.

At the haplotype level, each population exhibited a large number of unique haplotypes (Table 2), with only two shared lineages. However, the observed F_{ST} between the populations was rather low (0.029) and simulations conducted here demonstrate that under varying conditions of effective female population size and mutation rate the observed F_{ST} is not outside the 95% range of expected values (see Fig. 6). Thus, at the haplotypic level, the individuals buried at Conchopata cannot be excluded as being directly ancestral on the direct maternal line to those post-Wari individuals interred at Huari. However, given the discussion above concerning genetic continuity across much of the Andes during the Middle Horizon, we must also investigate whether this observation holds at the haplotypic level. In this case, comparative mtDNA sequence data are only available for the Middle Sicán sample. At the site of Sicán, 21 individuals were observed to exhibit 15 haplotypes belonging to one of the four major Native American haplogroups. Only one Sicán haplotype is shared by an individual at Conchopata (Concho36), that of the founder haplotype of Native American haplogroup B2. The genetic distance estimated between Sicán and Conchopata (F_{ST} = 0.055), while greater than that observed between Conchopata and Huari, is not significantly different (P = 0.065) than the expectation of no difference between the populations, evaluated with 10,000 permutation of the data as calculated in Arlequin 2.0 (Schneider et al., 2000). As discussed earlier, it appears that there was widespread genetic continuity in the Middle Horizon Andes at the haplotypic level as well.

CONCLUSIONS

This study highlights the strength of combining archaeological and osteological data with molecular data. Based on culture history alone, one might argue that major shifts in material culture, settlement patterns, incidences of violence, and bodily expressions of ethnic identity observed from Wari to post-Wari times was associated with an equally large shift in the mtDNA composition of the population (e.g., population replacement of maternal lineages). Ancient DNA evidence allows for such hypotheses to be directly tested, and in the case of the present study the cultural and political transformation that occurred from the Wari to post-Wari period was not concomitant with a major transformation in the mtDNA profile of the local communities. Rather, this time of political upheaval appears to have occurred against a backdrop of mtDNA continuity. Although it is possible that foreign groups with similar haplogroup distributions as those from Conchopata migrated to the former Wari imperial heartland, our analysis could not detect them. This scenario may be especially true in an area such as the Andes where, as discovered here, Middle Horizon populations have been demonstrated to reveal no significant mtDNA structure over great geographic distances. This means that future genetic studies aimed at reconstructing population history in this region of the world will require larger samples sizes and, if possible, the inclusion of genetic markers from the nuclear genome. Nonetheless, our study has shown that new populations with distinct haplogroup frequencies did not move into the former Wari imperial heartland. Thus, based on the current mtDNA data, there is no evidence to suggest the influx of genetically distinct females into the former Wari capital after Wari collapse.

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APPENDIX: EXAMPLE OF THE SERIAL SIMCOAL (EXCOFFIER ET AL., 2000; ANDERSON ET AL., 2005) INPUT FILE USED IN THIS STUDY FOR THE SIMULATION "SMALL, FAST." VARIABLE PARAMETERS OF EFFECTIVE FEMALE POPULATION SIZE WERE INPUT UNDER "DEME SIZES" AND VARIABLE MUTATION RATES WERE INPUT UNDER "MUTATION RATE."

```
//Input parameters aPeru 1) small, fast:
1 population with ancient DNA data
//Deme sizes:
100
//Sample sizes:
2 sample groups
17 0 0 1
8 27 0 0
//Growth rates:
0
//Number of migration matrices:
0
//Historical event:
0
//Mutation rate:
.0035
//Number of loci:
373
//Data type:
DNA 1
//Mutation rates gamma distribution shape parameter
0 0
```

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