Using Resampling Statistics to Test Male Interment Bias: Applications for Looted and Commingled Prehistoric Remains in Peru and the Reassessment of Neandertal Burials

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ABSTRACT

The taphonomic processes that cloud reconstruction of past cultural practices and paleodemography are complex and include the preservation of a specimen or the discovery of the site. There has long been a notion of sex related bias in Neandertal burials and it is an easy assumption to make any time the number of specimens attributed to one sex is greater than the other in any interred population. Resampling statistics can be used to test the likelihood that a specific sample is representative of the expected parent population. Here we examine statistical uncertainty in burial contexts using two examples—combined Neandertal burials and commingled remains from a single prehistoric tomb in Peru. The Peruvian tomb represents the problematic ossuary context that many bioarchaeologists encounter, while the Neandertal sample represents one of the most classic discussions of sex bias in our field's history. Using resampling statistics, we fail to reject the null hypothesis that Neandertal burials do not deviate significantly from the expectation of random draws from a population with a balanced sex ratio. The same finding is true for a prehistoric (AD 2000–1000) ossuary burial in highland Peru. The present data do not necessitate inference of male interment bias in Neandertals and suggest caution to bioarchaeologists interpreting commingled remains.

INTRODUCTION

Human remains are subject to a number of cultural processes that limit what is available in the archaeological record, taphonomic processes that limit what is preserved over time, and additional noise due to loss of information during excavation and analysis. In order to assess the paleodemography of a population, all of these factors must be accounted for before declaring the skeletal sample representative of past demographic and cultural parameters.

The Andean highlands are conducive to excellent preservation of prehistoric remains; however, most tombs have been looted since colonial times, resulting in the loss of grave goods and the information they provide (Gerdau-Radonic and Herrera 2010). Additionally, most tombs are ossuaries of multiple individuals. Disturbance by the looters eliminates the context necessary for identifying each individual. Estimations of minimum number of individuals (MNI) are rarely calculated with a bone suitable for identification of sex, such as the pelvis or the cranium, making it difficult to identify the sex ratio present in a commingled sample. Often, one sex is identified in higher numbers raising the question of a sex-related interment bias, which is difficult to answer given the disturbed state of the remains.

These issues are exacerbated in more ancient cases of human burial, such as Neandertals. Nonetheless, analyses of Neandertal skeletal remains have revealed several interesting aspects of Neandertal paleodemography. Analysis of Neandertal age profiles have revealed a high number of prime-aged adult deaths and correlated reduction in number of individuals living past prime reproductive years in Middle Paleolithic samples (Caspari and Lee 2004, 2005; Trinkaus 1995, 2011). While this apparent pattern may reflect the true demography of Neandertal populations, it may also reflect behavioral or cultural biases such as differential adult age-related burial (Trinkaus 1995).

Burial-related biases have been offered as explanation for the apparent surplus of males in Neandertal assemblages (Binford 1968; Harrold 1980). For example, Harrold (1980) performed an analysis of Middle and Upper Paleolithic Eurasian burial remains and noted a tendency towards over-representation of males in Middle Paleolithic burials. This pattern included a significant excess of grave furnishings included with males (eight of ten males, zero of seven females found with grave furnishings). While the apparent male interment bias in Harrold's sample is bolstered by the excess inclusion of grave furnishings with male burials, several biases could potentially affect this interpretation among Neandertal burials. For example, Har-

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TABLE 1. THE PERUVIAN SAMPLE FOR THIS STUDY: ADULT PELVIC REMAINS WITH IDENTIFIED SEX FROM THE HUALACAYÁN *MACHAY*.

Specimen and Code	Side	Sex	Age phase*	Approximate Age
Innominate 68	L	F	Aur: 4/5	~40 yrs
Innominate 562	L	F	Aur: 8 Todd: 9 S-B: 5	~50 yrs
Innominate 67	L	M	Aur: 3 Todd: 4 S-B: 2	~30 yrs
Innominate 71	L	M	Todd: 2 S-B: 5	~35 yrs
Innominate 204	L	M	Aur: 3 Todd: 2 S-B: 2	~25 yrs
Innominate 440	L	M	Aur: 3 Todd: 6 S-B: 4	~35 yrs
Innominate 563	L	M	Aur: 3 Todd: 7/8 S-B: 4	~35 yrs
Innominate 564	L	M	Aur: 5 Todd: 7 S-B: 3	~35 yrs
Pelvis 900	NA	M	NA	unknown

*Age phases from Buikstra and Ubelaker (1994). Aur = auricular surface age standards, Todd = Todd pubic symphysis age standards, S-B = Suchey-Brooks pubic symphasis age standards.

rold's analysis includes a total of eleven burials from the Skhūl and Qafzeh sites, which are traditionally classified as anatomically modern humans (McCown and Keith 1939; Vandermeersch 1977). Moreover, inter-observer biases and misclassification of sex in fragmentary Neandertal remains could constitute a substantial bias in these results (Weiss 1972).

Here, we reassess the evidence for a male interment bias in Neandertal samples and contextualize these results with a similar analysis of commingled and looted remains from a prehistoric Peruvian tomb (AD 200–1000). We analyze an example from northern Ancash, Peru, alongside one of the most discussed possibilities of male interment bias, the Neandertals, in order to demonstrate the practicality of resampling statistics for preliminary paleodemographic assessments. Further, we probabilistically incorporate into our resampling tests the potential variation induced by incorrect assessment of sex and burial status in these samples.

MATERIALS AND METHODS

PERU

The site of Hualcayán is a large archaeological complex situated in the northern Callejón de Huaylas valley, located in the highland Andes of north-central Peru. The occupation of the site ranges from the Late Preceramic Period to the Early Colonial Period (~2500 BC–AD 1600). Excavations in summer 2012 of an Early Intermediate Period through Middle Horizon (~AD 200–1000) *machay*-style tomb yielded the remains of over twenty individuals. The remains were commingled in the tomb's ancient use and have been looted in recent years. The individuals represented in the *machay* range in age from infant to old adult. There are only nine adults represented by pelvic remains sufficient for estimating sex by morphological differences. The pelvic remains for the left *os coxae* were identified as two female and seven male (Table 1).

TABLE 2. THE NEANDERTAL SAMPLE FOR THIS STUDY: INDIVIDUALS WITH IDENTIFIED SEX FROM KNOWN NEANDERTAL BURIALS.

Specimen	Sex	Source
La Chapelle	M	Trinkaus 1980
La Ferrassie 1	M	Trinkaus 1980
La Ferrassie 2	F	Trinkaus 1980
La Quina H5	F	Trinkaus 1980
Tabun 1	F	Trinkaus 1980
Amud 1	M	Trinkaus 1980
Shanidar 1	M	Trinkaus 1980
Shanidar 2	M	Trinkaus 1980
Shanidar 3	M	Trinkaus 1980
Shanidar 4	M	Trinkaus 1980
Shanidar 5	M	Trinkaus 1980
Shanidar 6	F	Trinkaus 1980
Shanidar 8	F	Trinkaus 1980
Neanderthal 1	M	Trinkaus 1980
Spy 1 cranium	M	Wolpoff 1999
Spy 2 cranium	M	Wolpoff 1999
St. Cesaire	M	Trinkaus 1999
Regourdou	M	Volpato et al 2012
Palomas SP-96	F	Walker et al 2012
Kebara 2	M	Rak and Arensburg 1987
Males	14	
Females	6	

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Table 2 lists the adult Neandertal remains thought to be excavated from burials in both Europe and the Levant. The site of Shanidar in Iraq has the largest number of adults from a single Neandertal burial site with five males and two females. We consider twelve sites totaling twenty individuals found in burials, fourteen males and six females. Identification of sex for the Neandertals is based on Trinkaus (1980), with the addition of St. Césaire (Trinkaus 1999), Regourdou (Volpato et al. 2012), and Palomas (Walker et al. 2012), and with the exception of Spy 2. According to Wolpoff (1999), the Spy 1 cranium is larger than the cranium of Spy 2 and generally regarded as male, but the comparisons do not reflect differences normally considered sexually dimorphic and thus he considers Spy 1 a second male. The postcranial skeletons of Spy 1 and Spy 2 do not necessarily correspond to the crania of the same numbers due to the mixed nature of the remains. Trinkaus (1980) concluded that Spy 2 postcrania were in the high end of the male range of variation and Spy 1 was in the middle of the female range. As Trinkaus (1978) demonstrated that there are three adult individuals represented in the Spy sample with no way to match crania to postcrania, we have the choice for our study to use cranial features or postcranial articular dimensions for sex estimation. Since the remains of the site are best known by the two crania, we have chosen to use those and designate both individuals as male.

RESAMPLING STATISTICS

We used custom Python (3.3.3) scripts to perform resampling tests. For our tests we define the sex ratio as the number of males divided by the total number of individuals. We utilize a resampling test to address the following null hypothesis: (H0) The number of males in a single sample does not deviate significantly from the expectation of unbiased interment (males and females interred with equal probability $\{\Theta = 0.5\}$). Additionally, we incorporate uncertainty in both the assessment of sex in the sample and interpretation of the remains as a burial into this resampling framework (Figure 1).

We examine this hypothesis in the Neandertal and *machay* samples. For a sample of N fossil individuals—9 for the *machay* and 20 for Neandertals in burial contexts—we randomly sample N individuals, each with a 50% chance of being male. Next, we randomly remove individuals from this sample with probability b, where b is the likelihood

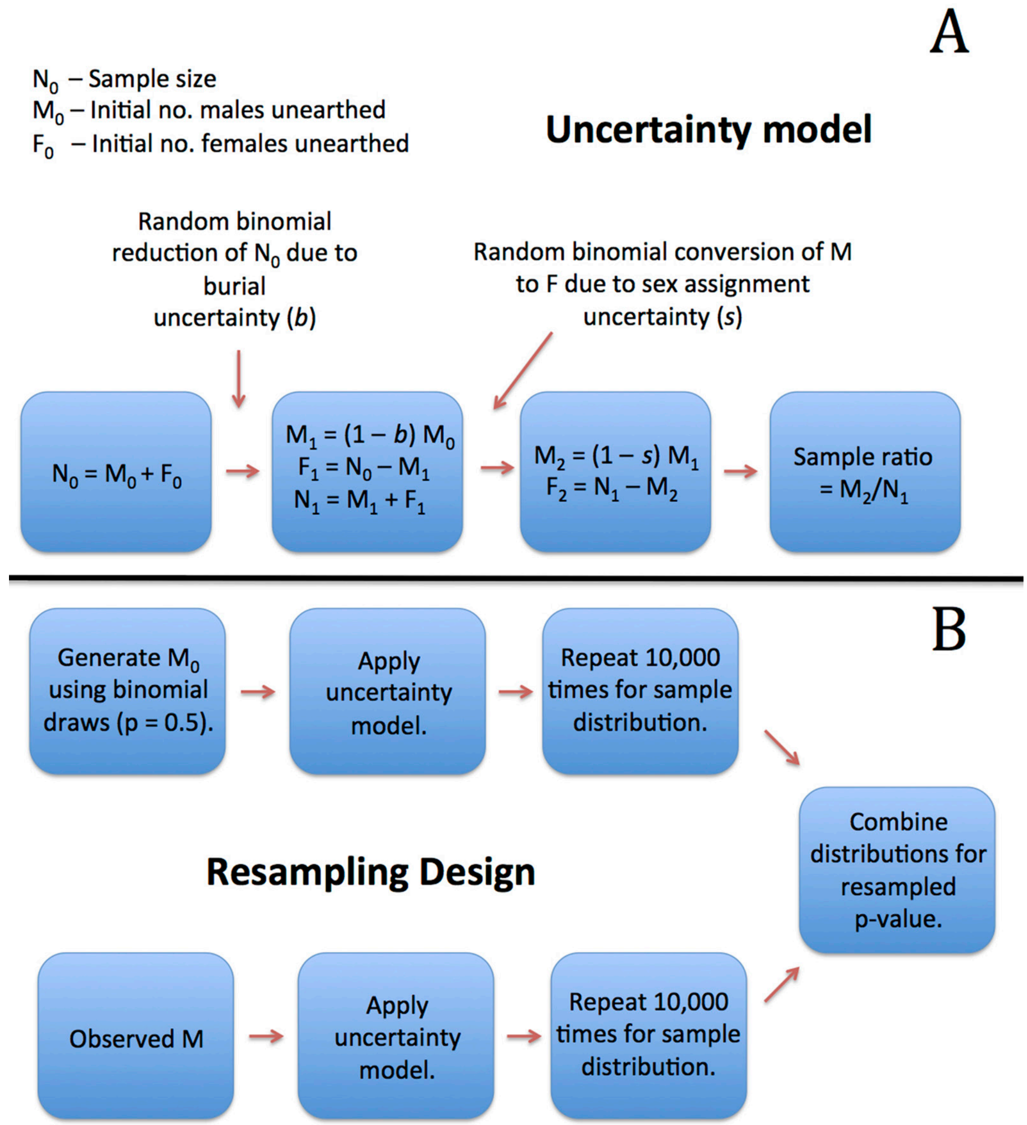

Figure 1. Illustration of Uncertainty Model and Resampling Method. Panel A illustrates the application of sex- and burial- uncertainty to an initial sample of males and females. Panel B illustrates resampling method as applied to random samples and observed samples. Final p-value results from the fraction of the distribution from the observed sample that falls above the 95th quantile of the null distribution.

that an individual is not a true burial. We then randomly convert males in the remaining sample to females with probability *s*, where *s* represents the likelihood of mis-assignment of male sex to a true female individual.

Subsequently, we count the number of males in this randomized sample and measure the absolute difference between the observed and expected (N/2) number of males. We repeat this procedure for 10,000 trials to create a null sample distribution. From this distribution we calculate a critical value (the value at which an observed sample of size N would be statistically significant (α=0.05). We then repeat this procedure on our observed number of males to generate a distribution of 10,000 observed values accounting for sex- and burial-uncertainty. Finally, we compare these two distributions to calculate an empirical p-value as the fraction of our observed distribution that falls below the critical value of the null distribution.

The null hypothesis is rejected if the observed number of males in a sample is greater than two standard deviations from the mean of the resampled null distribution (i.e., $p<0.05$). We assess the impact of *b* and *s* ranging from 0.0 to 0.25 in five percent increments.

We note that in the special case where $b = s = 0$, there is no variation in the observed test statistic. Therefore, our empirical p-value collapses to either 0 or 1 corresponding to the p-value of a two-tailed binomial test falling below or above 0.05, respectively. However, addressing the uncertainty of sex-determination and burial status requires permutation-based (resampling) statistics due to the lack of uncertainty in the underlying statistical distribution assumed in parametric tests.

TABLE 3. QUANTITATIVE RESULTS FROM RESAMPLING TESTS.

Sample	Neandertals	Andean
Observed No. Males	14	7
Sample Size	20	9
p-values*		
***s*=0.0; *b*=0.0**	1.0	1.0
***s*=0.05; *b*=0.0**	1.0	1.0
***s*=0.10; *b*=0.0**	1.0	1.0
***s*=0.15; *b*=0.0**	1.0	0.9999
***s*=0.20; *b*=0.0**	1.0	1.0
***s*=0.25; *b*=0.0**	1.0	1.0
***s*=0.0; *b*=0.05**	0.9814	1.0
***s*=0.0; *b*=0.10**	0.9679	1.0
***s*=0.0; *b*=0.15**	0.9609	1.0
***s*=0.0; *b*=0.20**	0.9662	1.0
***s*=0.0; *b*=0.25**	0.9684	1.0
***s*=0.05; *b*=0.05**	0.9911	1.0
***s*=0.10; *b*=0.10**	0.9999	1.0
***s*=0.15; *b*=0.15**	1.0	1.0
***s*=0.20; *b*=0.20**	0.9999	0.9996
***s*=0.25; *b*=0.25**	0.9994	0.9997

*p-value calculated as the fraction of adjusted sample distribution greater than the critical value determined from the resampled null (θ=0.5) distribution.

POWER ANALYSIS

We conducted *a priori* power tests to determine statistical power (1–β), where β is the probability of type II error (false-negative rate), of our tests. We set our significance level (α) at 0.05. We assessed the power of each of our tests to reject H0 given the true male interment fraction is Θ. We assessed power across the full range of Θ from 0.0 to 1.0 in 1% increments (Supplementary Figures 1–6). We note that for our limited sample sizes (as are common in analyses of archaeological remains), we have little power to reject a false H0 when the true alternative interment parameter (Θ) only moderately deviates from H0. For example, if the sex ratio in the Neandertal sample (Θ =0.7) represents the true ratio at which males were interred in Neandertal populations, we do not have adequate statistical power (at least 0.8) to reject the null (Θ=0.5). However, we have adequate power to strongly reject H0 when the true interment parameter (Θ) deviates greatly from 0.5. In contrast, we have no power to confidently detect a significant deviation from the null in a sample as small as the *machay*.

RESULTS AND DISCUSSION

PERU

As mentioned above, a sample of nine is simply too small to detect a significant deviation in sample sex-ratio from the null expectation. Therefore, despite the high observed sample sex-ratio (0.78), we fail to reject the null hypothesis for all tests on the *machay* sample (Supplementary Figures 23–38, Table 3).

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Similarly, the Neandertal sample (N=20) is also too small to reject the null, assuming the alternative hypothesis is less than 0.8. Allowing for uncertainty in sex-estimation and burial status decreases (and for high uncertainty) removes our power to reject any alternative hypothesis. Therefore it is also not surprising that we strongly fail to reject the null hypothesis on all accounts (Supplementary Figures 7–22, see Table 3).

IMPACT OF UNCERTAINTY IN SEX- AND BURIAL- STATUS

Our simulations illustrate that the effect of accounting for possible uncertainty in sex- and burial- status of samples is to increase the variance in the underlying null distribution. Accounting for such uncertainty decreases statistical power to reject the null across the full range of alternative hypotheses (Θ [0,1]), thereby placing a higher burden on small samples. The impact of this result is that the sample sizes necessary to demonstrate a significant deviation from the null expectation and account for uncertainty need to be greater than sample sizes necessary to demonstrate this using the binomial probability (assuming no uncertainty in the sample).

IMPLICATIONS OF RESULTS CONSIDERING LOW POWER

In fields such as paleoanthropology and archaeology that are often plagued by small sample sizes, resampling statistics are a valuable tool for testing whether excavated remains fall outside of chance expectations. We fail to reject the null hypothesis in both the case of the Andean *machay* and the Neandertals. However, considering a power analysis on each of the samples in this study (see Supplementary Figures 1-6), we note that the currently available sample sizes are simply not large enough to demonstrate a bias with statistical significance. Assuming no bias in sex-estimation or burial status, we can confidently say that the true Neandertal interment sex-ratio did not likely exceed 0.8. If it did, our test would have detected a significant deviation from the null.

FAILURE TO REJECT THE NULL DOES NOT IMPLY UNREPRESENTATIVENESS

Our results demonstrate that there is no statistical evidence for an interment bias in either of our samples. However, we also demonstrate that our failure to reject the null may be due to low statistical power given our small sample sizes. Although our samples are underpowered to confidently detect deviations from the null, it remains that these samples are unrepresentative of the expected population sex-ratio of Neandertals, which theoretically was near 0.5. Therefore, we may also ask how large our samples would need to be in order for the observed sample sex-ratios to significantly deviate from the null. Assuming no sample uncertainty, doubling the Neandertal burial sample size (to N=40) and holding the sex-ratio constant would provide enough power to reject the null (Supplementary Figure 39). Applying even mild uncertainty in sex-estimation (s=0.05) would require a quadrupled sample size (N=80) to produce a significant result (Supplementary Figures 40–42).

POSSIBILITY OF SEX- AND BURIAL- UNCERTAINTY

In addition to sample size, another potentially major source of bias is sex designation. The estimation of sex for Neandertal individuals is not entirely agreed upon in the field. Working solely with burials, the pelvic remains available for sex assessment are limited (Males: Amud 1, La Chapelle-aux-Saints 1, La Ferrassie 1, Shanidar 1, Shanidar 3, Shanidar 4, and Kebara; Females: La Ferassie 2, Tabun C1) leaving the determinations for the other individuals to be based on other parts of the skeleton. The other Shanidar individuals sort more easily since the sample is relatively large, but we do not have the same benefit with other skeletons. The Spy Neandertals are notoriously problematic. We settled on designating both individuals as male since the variation between the two is not clearly dimorphic and for our purposes male is the more conservative estimate. Trinkaus does not provide an estimate for Regourdou 1 because the postcranial remains are too intermediate, however Volpato and colleagues (2012) argue the skeleton is male based on anatomical proportions, the alae in the superior sacrum, and canine breadth. The criteria outlined by Smith (1980) to assign sex to crania suggest La Quina 5 is a male but we used Trinkaus' (1980) postcranial assessment that the individual is female because it was the most consistent with the rest of our designations.

Archaeological assessment of burial status is another possible source of error in addressing the hypothesis of sex-bias in interred samples. We define a sample of burials based on broad consensus in the field; however, the Neandertal practice of burial in general has its opponents. For example, Gargett (1989, 1999) asserts that every instance of Neandertal burial could be interpreted as natural processes such as cave collapse. Although Gargett's work was controversial, such work has encouraged scholars to re-examine evidence of burial. Most scholars are more confident in the existence of the practice, as evidenced by the comments to Gargett's 1989 paper. Current research continues to address the topic, most recently by identification of the anthropogenic burial pit at La Chapelle-aux-Saints (Rendu et al. 2014). Just as misdiagnosed sex can change the significance of the results, it is also true that a misdesignated burial could change the outcome of this work. The appearance of male bias in burials could be the result of not having the full picture of who was actually buried. In some cases burials may not appear different from natural processes such as cave collapse and could skew our records of Neandertal burial practices, particularly if true female burials are by chance mistaken as non-burials.

The Andean sample presents less of these sources of error since it is clear that every individual was intentionally interred in the *machay* structure and sex determinations were obtained from pelvic material. However, Weiss (1972) notes a tendency for an overestimate of males in most skeletal samples, even when pelvic material is preserved and sophisticated analyses are used. One of the authors (JL) personally estimated sex in the Andean sample, and although sex was only recorded when confident with the designation, it is still an estimate and the same bias that plagues the eye of most osteologists could easily affect this analysis as well.

FUTURE DIRECTIONS

While we may never have much larger samples of Neandertal burials at our disposal, we may be able to increase our power to examine the question of interment bias by direct comparison to non-burial archaeological samples. For example, in the case of Neandertals, we may reframe our question and ask how likely it is that the burial and non-burial samples were drawn with equal probability from source populations with an equal sex ratio (determined by combining the two samples). If we assume (optimistically) that we have a sample of 50 Neandertals from non-burial contexts with reliable sex-designations at our disposal, we could demonstrate, using a resampling test, a significant

($p<0.05$) bias between our burial sample of 20 and a non-burial sample of 50 if the non-burial sample was slightly female biased (28 females). Therefore, a comprehensive assessment of sex ratio in Neandertals is needed to facilitate direct comparison between burial and non-burial samples to further assess the possibility of a male interment bias.

CONCLUSIONS

We have demonstrated that an intentional burial bias is not necessary to explain the surplus of males in Neandertal burials due to small sample sizes. While we have established that it is not necessary to invoke a cultural mechanism such as interment bias to explain the male bias in Neandertal burials, we note that our tests are subject to reduced power from diminutive sample sizes.

However, it remains true that the currently known sample of Neandertals with confident sex assignment based on the pelvis (see Trinkaus [1980] and above) consists of a greater number of males than females. It will be important to understand the full extent of this surplus of males in the Neandertal record to improve estimates of aspects of Neandertal biology such as body mass and brain size, which rely on an accurate assessment of sex. This work suggests that researchers should continue to discuss and analyze potential reasons for unrepresentative sex-ratios in archaeological samples and that caution should be taken to not overstate the potential behavioral implications of these sex-ratios until sufficiently large samples can be gathered to address such questions.

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