Beyond Size: the potential of a geometric morphometric analysis of shape and form for the determination of sex in hand stencils in rock art

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Abstract

Hand stencils are some of the most enduring images in Upper Palaeolithic rock art sites across the world; the earliest have been dated to over 40 Kya in Sulawesi and 37 Kya in Europe. The analysis of these marks may permit us to know more about who was involved in the making the prehistoric images as well as expanding the literature on the evolution of human behaviour. A number of researchers have previously attempted to identify the sex of the makers of Upper Palaeolithic hand stencils using methods based on hand size and digit length ratios obtained from digital or photo-based images of modern reference samples. Most recent analyses report that it was probably mostly females that produced the majority of prehistoric hand stencils. Taken together, however, these studies generate contrasting and probably incompatible interpretations. In this study we critically review where we currently stand with methods of sexing the makers of hand stencils and the problems for the interpretation of hand markings of Palaeolithic age. We then present the results of a new method of predicting the sex of individuals from their hand stencils using a geometric morphometric approach that detects sexual differences in hand shape and hand form (size and shape). The method has the additional advantage of being able to detect these differences in both complete, as well as partial hand stencils. Finally we urge researchers to test this method on other ethnic groups and populations and consider ways of combining efforts towards a common goal of developing a robust, predictive methodology based on diverse modern samples before it is applied to Upper Palaeolithic hand stencils.

Key words: Palaeolithic, cave art, sex identification

Highlights

- A method is presented using geometric morphometrics to identify the sex of the makers of hand stencils using a modern sample

- Using the form of the stencil, we were able to correctly predict sex in over 90% of our sample

- The most sexually dimorphic area of the hand was the palm, which potentially allows for the prediction of sex from hand stencils with digits missing.

- We advocate collaboration between research groups in developing methods to assess the sex of the makers of hand stencils in contemporary populations before applying methods to Palaeolithic images

1. Introduction

Images of the human hand provide us with some of the earliest, most abundant and most enduring images in rock art (Pettitt et al., 2014, 2015; García-Diez et al., 2015). They have been recorded at sites across the Americas, Africa, Arabia, Australia, East and South Asia and Europe (e.g., Aubert et al., 2014; Clottes, 2010; Chazine, 2005). In some cases hand images are pecked into the rock (see Clottes, 2010), but the most common forms require the use of paints to create prints or stencils. Hand prints are made when a hand coated with paint or pigment is pressed against a surface, leaving a direct image; a stencil occurs when a clean hand is placed directly against a surface and paint or pigment is applied over the top, such that when the hand is removed a negative impression of its presence remains (Pettitt et al., 2015). In contrast to pecked hand images, hand prints and hand stencils necessarily preserve a record of the original size and shape of a real hand and these images, therefore, can be directly related to an/the individual actively involved in the prehistoric image-making process.

Hand images from sites around the world are likely to span many thousands of years in age, with some examples of images having been produced relatively recently (in Australia, Africa) and others much longer ago (Aubert et al., 2014; Pettitt et al. 2015). Possibly the oldest surviving corpus of images comes from Europe, where most hand images are likely to date before 20,000 years ago (Snow, 2013). In Western Europe, there are thirty-eight sites of accepted Palaeolithic age with preserved images of human hands on their walls; nearly 1000 images in total (Groenen, 2011; Snow, 2006). The number of images varies greatly between sites. Most sites have a small number of images, a few have tens of such images (e.g., El Castillo, Maltraveiso, Rouffignac), and a very few have hundreds of hand images (e.g., Gargas, Chauvet and Cosquer) (Pettitt et al. 2015). In caves with the largest number of hand images, many of these images are partial, with missing digits or digits that are considerably shorter than they must have been (Leroi-Gouran and Michelson, 1986).

There is no generally accepted explanation for the making of these images in Palaeolithic times. Early ideas have included enjoyment, hunting magic, accidental marking, and some form of visual plea to the heavens (see Ucko and Rosenfeld, 1967), whilst more recently they have been linked to shamanistic practices (Lewis-Williams, 2002; Clottes and Lewis-Williams, 1996) or markings made by adolescent males perhaps during rites of passage (Guthrie, 2005). Images of partial hands have been interpreted as evidence of disease or mutilation (Janssens, 1957, though see Hooper, 1980; Wildgoose et al., 1982 for a re-assessment) or as forms of sign language (Leroi-Gourhan, 1967; Pradel, 1975, though see Rouillon, 2006 for a counter argument). Finally, a recent study has shown that hand images appear to have been deliberately placed in association with specific characteristics of the walls or geological features indicating that the topography of the cave walls may have been important in making these images meaningful to contemporary populations (Pettitt et al., 2014).

Ethnographic accounts, however, suggest that hand images were produced within hunter-gatherer societies in the context of a range of different activities. Using the Australian Aboriginal literature, Moore (1979) notes that hand images were made for the purposes of memorialisation of a person or a visit, to mark the number and direction of persons passing a place, as the signature of an artist, as a form of message to spirit ancestors and so on. Gunn (2006), also reviewing the Australian literature, adds that hand images might be made to mark a visit to a place, or to make a claim to an area of land. From literature describing groups in the South-western Cape of Southern Africa, Manhire (1998) suggests that hand stencils might have been made during curing ceremonies, whilst the smaller sub-adult-size hand stencils might have been made as part of initiation ceremonies. It is clear from the ethnographic literature, therefore, that we should expect hand images in mobile societies to have been made by both men and women, and by individuals of all ages according to the different places and activities that formed the context for image-making. Importantly, although the ethnographic literature highlights many different potential meanings and purposes for these images, knowledge of who was present might allow different interpretations to be advanced or excluded. The correct identification of the sex and age of hand images, therefore, is a necessary first step to understanding this most common of Palaeolithic image forms.

2. The identification of sex in hand images using size data

In recent years there have been several studies that have attempted to identify the sex, and sometimes age, of hands preserved as stencils in caves or open rock art sites by comparing size data extracted from the images (e.g., Groenen, 1988; Gunn, 2006; Guthrie, 2005; Mackie, 2015; Snow, 2006, 2013; see Fig 1; Table 1). Some of these studies have also used digit ratios that have been shown to be a predictor of sex in some circumstances (Kanchan et al., 2008, but see Voracek, 2009). Researchers have then made comparisons between the dimensions from prehistoric images and the data collected for the same measures from a series of contemporary populations (Table 1). Some of these attempts have, however, produced quite contradictory results that necessarily lead to different possible interpretations of the meaning or purpose of the original images. For example, in his examination of hand stencil images from a number of European cave art sites Guthrie (2005) argues that most hand images are those of males with adolescents in the majority; however, Snow (2006, 2013), examining stencils from some of the very same sites as Guthrie, primarily identifies females. Both these studies base their analyses on robust comparisons with a large reference sample of modern hand data taken from different populations that they argue are good analogues (on the basis of genetic continuity) for populations living in Western Europe at the time when hand images were made.

Figure 1. Size dimensions used in previous studies (also see Table 1); A) Flood, 1987; B) Groenen, 1988; C) Guthrie, 2005; D) Gunn, 2006; E) Snow, 2006, 2013; F) Mackie, 2015.

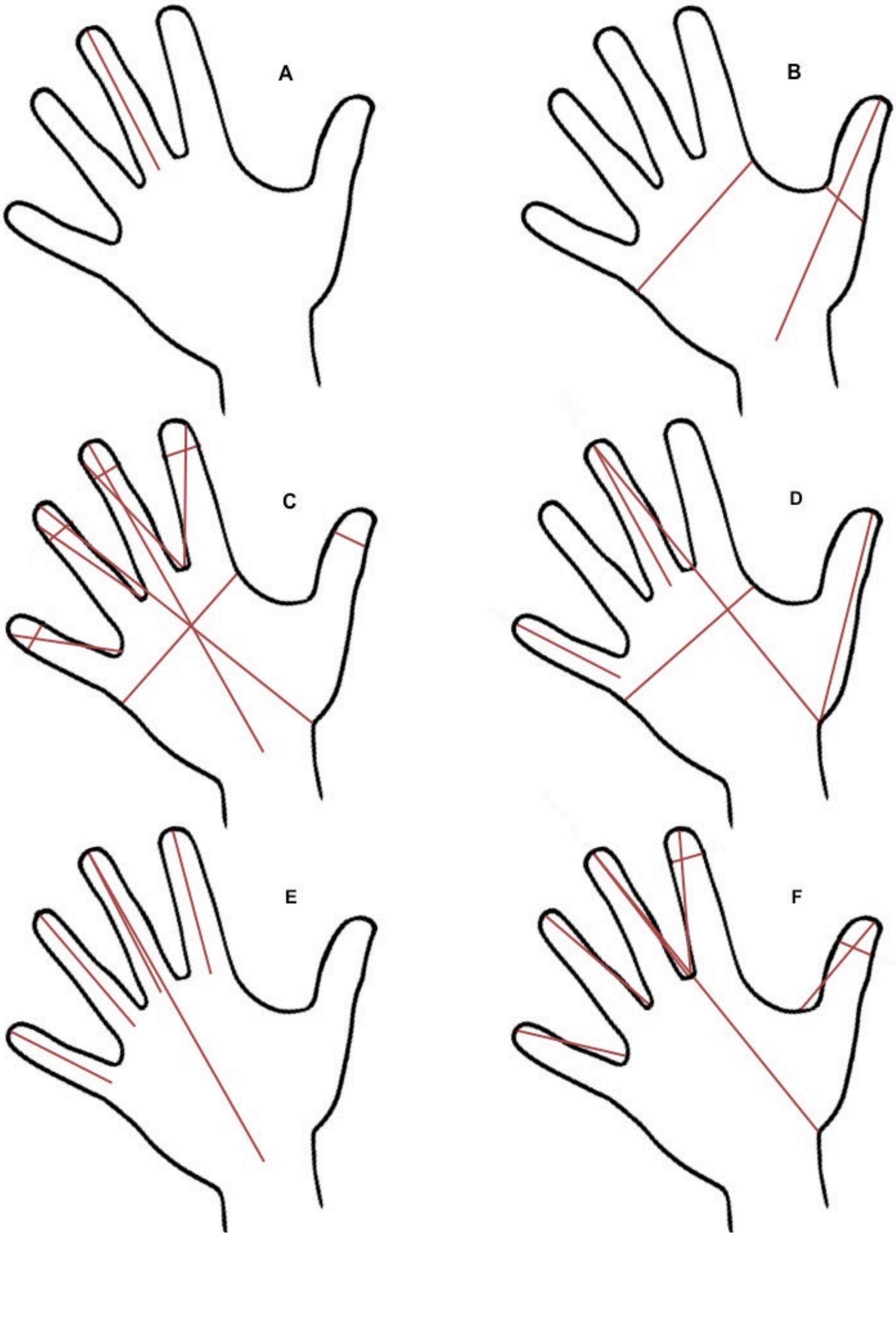


Table 1. Summary of previous studies that have attempted to sex the makers of ancient hand stencils.

Authors	Prehistoric site	Reference sample (RS)	Method	Interpretation of prehistoric hand stencils	
Flood 1987	Koolburra Plateau, Cape York, Australia	Modern Aboriginal sample (Abbie, 1975).	Flood used middle finger length from prehistoric stencils to devise 5 size classes of hand size. These measurements were then compared to the RS.	It was concluded that it is not possible to use this method to assign sex or age to the makers of hand stencils. However, she proposed that very large hand stencils were likely to be male.	Large stencils = ?♂
Groenen 1988	Gargas and de Tibiran Cave, Hautes-Pyrenees, France	The hands of 152 males and females aged from 19-13 from Brussels, Belgium.	4 measures of hand size for 55 Palaeolithic hand stencils were compared to the RS	Clear visual overlap in size dimensions between the modern sample and the prehistoric hands indicated individuals of both sexes and of broad range of ages took part in creating hand stencils in Gargas and de Tibiran Caves.	♂♀
McDonald 1995	Great Makeral and Yengo 1, Sydney Basin, Australia	Modern Aboriginal sample (Abbie, 1975).	McDonald compared hand size of prehistoric stencils (measurements were not described in the article) and compared the RS.	It was concluded that discerning gender (sex) from hand stencils would be difficult as male and female hands overlapped in size by 1 cm. However, McDonald proposed that large hand stencils were likely to be male, especially if these were stencilled with male-related tools such as boomerangs She speculated that hands with amputated 5th digits were likely to be female as this mutilation is a cultural indicator restricted to females in some local Aboriginal groups.	?♂♀, using other features
Guthrie 2005	Unspecified set of Palaeolithic cave sites in Europe	700 hands of males and females of west European descent sampled at yearly intervals from 5 through to 19.	Guthrie analysed 201 prehistoric hand stencils using univariate analyses based upon 12 linear measurements of the RS.	Guthrie was able to assess that 169 of the prehistoric hand stencils were male hands and 32 were female. Guthrie argues that the majority of these were the stencils of adolescent males.	♂♀, mostly adolescent males

Table 1 continued.

Authors	Prehistoric site	Reference sample (RS)	Method	Interpretation of prehistoric hand stencils	
Gunn 2006	Kulpi Mara, Central Australia and Reedy's Rockhole and Poona shelter, Murchison region of south-west Western Australia	Modern Aboriginal sample	Gunn carried out an experimental study on a modern sample of hand stencils and the hands that had produced them (sample size unknown). All the measurements taken from the hand stencils were larger than the real hands; except middle finger length, which tended to be smaller due to bleeding of the pigment. Nevertheless he advocated using middle finger length from stencils when interpreting data in relation to sex.	Gunn attempted to apply the measurements to hand stencils from two rock art sites in Australia (Kulpi Mara = 53 stencils; Reedy's Rockhole and Poona shelter = 92 stencils). However, he concluded that he was unable to assess sex or age from hand stencils with any degree of certainty.	?♂♀
Snow 2006, 2013	Snow 2006; 3 Palaeolithic hand stencils each from 3 different French cave sites (Abri du Poisson, Les Combarelles, Font de Gaume), plus 3 stencils from a replica of Pech-Merle Cave. Snow 2013; 32 hand stencils from 8 caves in France and Spain, including the caves from the 2006 study.	222 scanned hands from 111 males (n=54) and females (n=57) of Northern European descent.	Snow's analysis is based on five hand measures from individuals in the RS. He combined two approaches, firstly predictive formulae based on hand length and digit lengths (digits 2 and 5) were taken from 4 repeated scans from modern-day individuals' left and right hands. This enabled statistical constants to be established which were placed within an algorithm to predict sex from hand/digit measurements of males and females (79% accuracy). Comparisons of digit ratios (2D:4D) between the Palaeolithic stencils and modern scanned hands served as an additional discriminator. However, the accuracy for digit ratio was only 59%.	In the 2006 study, 4 stencils were identified as female and 2 as male. In the 2013 analysis of the expanded sample, 23 of the stencils were identified as female and 9 as male. In his studies, Snow found that sexual dimorphism in hands appeared to have been greater in the Palaeolithic.	♂♀, mostly females

Table 2 continued.

Authors	Prehistoric site	Reference sample (RS)	Method	Interpretation of prehistoric hand stencils	
Chazine & Noury 2006	Gua Masri II Cave, East Kalimantain, Borneo	The RS and the hand measurements upon which the software algorithms are based, are not described in the article. Values for digit ratios (2D:4D) are based upon a sample from Liverpool, UK (Manning, 1998).	A digital imaging software called Kalimain© (Noury, 2005) was used to sex the makers of prehistoric hand stencils. The method appears to use software that analyses both hand size and digit ratio to differentiate sex.	Out of 140 hands, only a quarter were deemed suitable for the application of the sexing software; 16 were identified as female, 17 as male. 2 were unable to be assessed using hand size, although 2D:4D (Liverpool RS) suggested the hands were male.	♂♀
Wang et al. 2010	Snow 2006; 3 Palaeolithic hand stencils each from 3 different French cave sites (Abri du Poisson, Les Combarelles, Font de Gaume), plus 3 stencils from a replica of Pech-Merle Cave.	Scanned hand images (17 males; 17 females; Snow, 2006) and 107 photographs (51 male and 56 female) of handprints in concrete of famous people taken from the Chinese Theatre in Hollywood, Los Angeles, USA	Wang and co-workers applied recognition software to a set of hand scans from contemporary people. The software used a size-invariant technique to analyse the images of known sex to create an outline of the hand and segments. 125 hand scans were used in 10 rounds of cross validation to train the software after 50 were randomly selected and kept as the validation dataset. Support Vector Machine was then trained to recognize sex differences in the hands. Accuracy of 75% was achieved.	When the method was applied to images of Palaeolithic hand stencils (n=6; Snow, 2006), two of the cave hand stencils previously identified as female by Snow (2006) were identified as male. Their results (based on the sample from Snow 2006) also confirmed Snow's proposal that hand sexual dimorphism was more pronounced in the past, based on the tendency for the Palaeolithic hand stencil data to cluster around the extremes of the scale-continuum based on modern hand scans.	♂♀
Pettitt et al. 2014	6 hand stencils from El Castillio and La Garma Caves, Cantabria, Spain	Snow 2006	Snow 2006	Snow's (2006) method was applied to 6 hand stencils; 4 were identified as female and 2 as male.	♂♀

Table 1 continued.

Authors	Prehistoric site	Reference sample (RS)	Method	Interpretation of prehistoric hand stencils	
Mackie 2015	3 prehistoric Indian rock art sites(48J03; 48J04; 48J06) located in Johnson County, Wyoming, USA	271 hand stencils of modern males (n=93) and females (n=178). Nearly half (46%) were aged 18 or younger.	18 points were mapped on to each digitally scanned stencil, which helped to guide linear measurements of the hand. Based upon these measurements a series of equations were formulated. A 75% level of accuracy was reported for the sexing equations. Using close range photogrammetry, measurements from 78 prehistoric hand stencils were analysed, but only 25 were complete enough to be sexed.	7 prehistoric stencils were identified as female, 13 as male and 5 were indeterminate. It was concluded that individuals of both sexes and a broad range of ages took part in creating hand stencils at these locations. 2D:4D data did not suggest there were higher levels of sexual dimorphism in this prehistoric population.	♂♀

A major problem with these studies is the reliance on size data to identify sex. The use of size data alone is made much more difficult by the fact that even though the average size of adult male hands is greater than female hands, within any population there is a varying degree of overlap in size between the two (Galeta et al., 2014; Gunn, 2006; Králík et al., 2014). Furthermore this overlap exists, not only in the comparison of adult hands, but also between age cohorts of different sexes due to the different timings of male and female growth spurts (Guthrie, 2005). This general problem of hand size overlap between sexes has already been recognised for the use of size data alone to sex hand images (Flood, 1987; Galeta et al., 2014; Gunn, 2006; McDonald, 1995), as well as between sexes and across populations in the use of digit ratios for the same purpose (Nelson et al., 2006).

The use of size data is not the only problem with published archaeological studies. Whilst noting that Guthrie and Snow came to strikingly different conclusions about the sex most commonly represented in images in samples from the same prehistoric sites, we remain unable to evaluate their interpretations since both Snow and Guthrie, and most other analysts, use slightly different measurements (Fig. 1). Only Snow has identified the specific stencils that he examined so that in future we might be able to evaluate different methods when applied to the same original image. As noted above, many hand images, especially from caves with the largest collections, are partial representations, some with missing digits and many with missing basal features of the palm, and these images cannot be used in some approaches. A further difficulty has been highlighted by Gunn (2006) who has shown that different stencil images made using the same hand can differ in their size measures by up to 5mm due to the localised variations in the way the paint covered the hand.

Such archaeological approaches have to be understood in the context of biological variation in the human hand. Elsewhere hand outlines have been utilised in biometric studies for purposes of individuation and sex estimation in a forensic setting. In particular, shape variation in the hand has been classified using the length and width of the fingers, their curvatures, the relative location of these features, or the relative placement of the palm in relation to the digits; some of these classifiers have relied solely on geometric features based on linear chord distances, while others use hand silhouettes with or without geometric features, in an attempt to attribute a specific hand pattern or outline to an individual. Most methods require the capture and analysis of a significant number of chord distances or morphological features, ranging from a minimum of 16 basic descriptors to as many as 160 features often comprised of many tens of thousands of individual points. Whilst such methods are required in order to individuate a hand pattern as part of biometric security systems, such data-heavy methods are not required in order to attribute hand shape to biological sex, but a robust biologically-relevant statistical method must be applied which allows for both size and shape of hand morphology to be assessed.

3. A new approach to the identification of sex from hand images

It is clear that hand images potentially offer great opportunities for the assessment of the sex and age of individuals present at sites during moments of artistic creation. In order to realise the full potential of these images, however, we need to be able to assess the sex of the individual using methods that are not reliant on size data alone. Methods that can attribute sex in cases of partial images would also be desirable, and finally we need to use a set of data collected against a clearly defined set of landmark points on a hand image so that conflicting interpretations can be independently evaluated. If it proves possible to assess sex without reliance on size alone, it may then be possible to use relative size within a defined sex grouping to assess age and this will make it more possible to offer interpretations for activities that led to creation of these images.

The research presented here is the first attempt to utilise geometric morphometric techniques to quantify shape variation in the human hand, and consequently addresses the potential of this anatomical region for sex estimation. We evaluate the success of shape analysis in a large comparative collection of hand stencils based on data for the position of 19 landmark points on the hand. Geometric morphometrics (hereafter GMM) is the statistical analysis of form based on Cartesian landmark coordinates (Mitteroecker and Gunz 2009). Whilst the fundamental underpinnings of the discipline date back to the early 20th Century, it is only recently that modern computational and technological advances have allowed for the acquisition, processing, and analysis of shape variables that retain all of the geometric information contained within biological data. GMM techniques generally involve the capture of homologous landmarks, which can be defined as precise locations on biological specimens that hold some functional, structural, developmental, or evolutionary significance and are directly comparable between specimens. The locations of homologues can be recorded as two- or three-dimensional co-ordinates, which result in a spatial framework of the relative positions of the chosen landmarks in Euclidean shape space. However, whilst coordinate data retain the full geometry of the landmarks (and hence shape), they have proved more difficult to compare statistically than conventional linear dimensions, primarily for reasons of registration between configurations. To overcome this, GMM analyses allow for the extraction of shape differences between configurations with residual shape being defined as the geometric properties of an object invariant to orientation, location, and scale. Our analysis is based on a study of more than 130 hand stencil images collected from a population of both male and female adults. The stencil images were produced and recorded in a standard manner to minimise error that might relate to the technology of image-making. The landmark points used include points on the digits as well as the palm that can be located clearly in terms of the anatomy of the human hand. In our analysis we have also examined whether it is possible to differentiate sex in cases where digit data is missing by using the palmar surface only in sex estimation.

The aim of this biometric study was to develop a methodologically and statistically robust means for investigating the sex-based form of the human hand by studying the extent of morphological variation using true hand geometry within a sample population using a small suite of retained geometric landmarks. We suggest that this approach may have similar or greater discriminating power compared to other published methods whilst requiring fewer captured features in order to identify or verify sex from hand shape. Results indicate that shape, or shape and size, effectively enables the attribution of sex to a statistically significant degree, avoiding some of the problems within modern studies that rely on size data alone to identify sex. Furthermore, this study enables us to utilise shape analysis for sexing individuals even in cases where the images of hands are partial (i.e., with partial or missing digits), opening up the possibility of using a much bigger corpus of hand images from the largest sites.

4. Materials and methods

4.1. Permission and protocol

The research protocol was reviewed and the University of Liverpool's Committee on Research Ethics granted permission for the study. Potential participants were given an information sheet about the aims of the study, what they had to do and their rights to withdraw their information at any point in the data collection period. The information sheet clearly stated that people with injuries to the hands or fingers or disfigurements to the hands or fingers were excluded from the study. People known to have had allergic or skin reactions to water-based poster paints were also excluded.

4.2. Sample

Participants were recruited at the University of Liverpool (UK) and filled out a questions sheet asking their sex, age, height and ethnic group (Ethnic groups was ascertained using the standard classification of ethnicity used by the University of Liverpool's Human Resources Department). As the majority of the sample was Caucasian (94.7%); we classified individuals as Caucasian or non-Caucasian.

4.3. Data collection

4.3.1. Stencils

Stencils were collected from 132 individuals (the left and right hands of 53 males; 79 females). One hundred and twenty four participants were Caucasian and 7 were non-Caucasian (Asian); one individual did not reveal their ethnic origin. An A4 sheet of 80gsm cartridge paper was taped to the laboratory wall and participants placed their hands flat against the paper. Hands were sprayed with a diluted solution of poster paint (one part paint to three parts water) using a Wolf Powerplus electric spray gun. The spray gun was maintained at the distance of about 0.5 metres from the hand/wall; positioned posterior and lateral to just the participant's elbow. Hands were stencilled with the fingers open and both the left and the right hands of each participant were stencilled (one after the other) using the same process. Stencils were left to air dry.

4.3.2. Landmarks

Standardised digital images were captured of both left and right hands by scanning the paper-imaged stencils using a Packard Bell Diamond 1200 scanner and were saved in a jpeg format (300 dpi). A 10 cm scale was placed on the scanner. Nineteen two-dimensional landmarks were recovered from digital image capture followed by landmark acquisition. Each scan was loaded into TPSDig2 (Rolf, 2008) and 19 type II and III landmarks (following the definitions of Bookstein, 1991) were applied to each stencil (digital) images (see Fig. 2; Table 2) using an adaptation of an existing biometric protocol (Randolph-Quinney et al., 2010). The landmarks were chosen to reflect the position and proportions of the phalangeal rays with respect to the metacarpus and palmar base. Landmarks were also selected due to their repeatability, permanence, and ability to describe the proportions and overall morphology of the hand, and included the tips of, and webbing between, the five phalangeal rays. Landmarks (X-Y coordinates) were plotted and saved in the same order for each stencil (1-19; Fig. 2). Type II landmarks are mathematical points whose claimed homology is supported only by geometric evidence i.e., the sharpest curvature of a tooth or the tip of a digit. Type III landmarks have at least one deficient coordinate, for instance the geometric minima or maxima, such as either end of the longest diameter, or the bottom or a concavity (i.e., between the web space of the digits). Both types, though less geometrically efficient than Type I landmarks, carry significant information regarding homology, and are thus biologically highly-relevant as recognised in published studies.

Figure 2. Landmarks for each stencil (see Table 2).

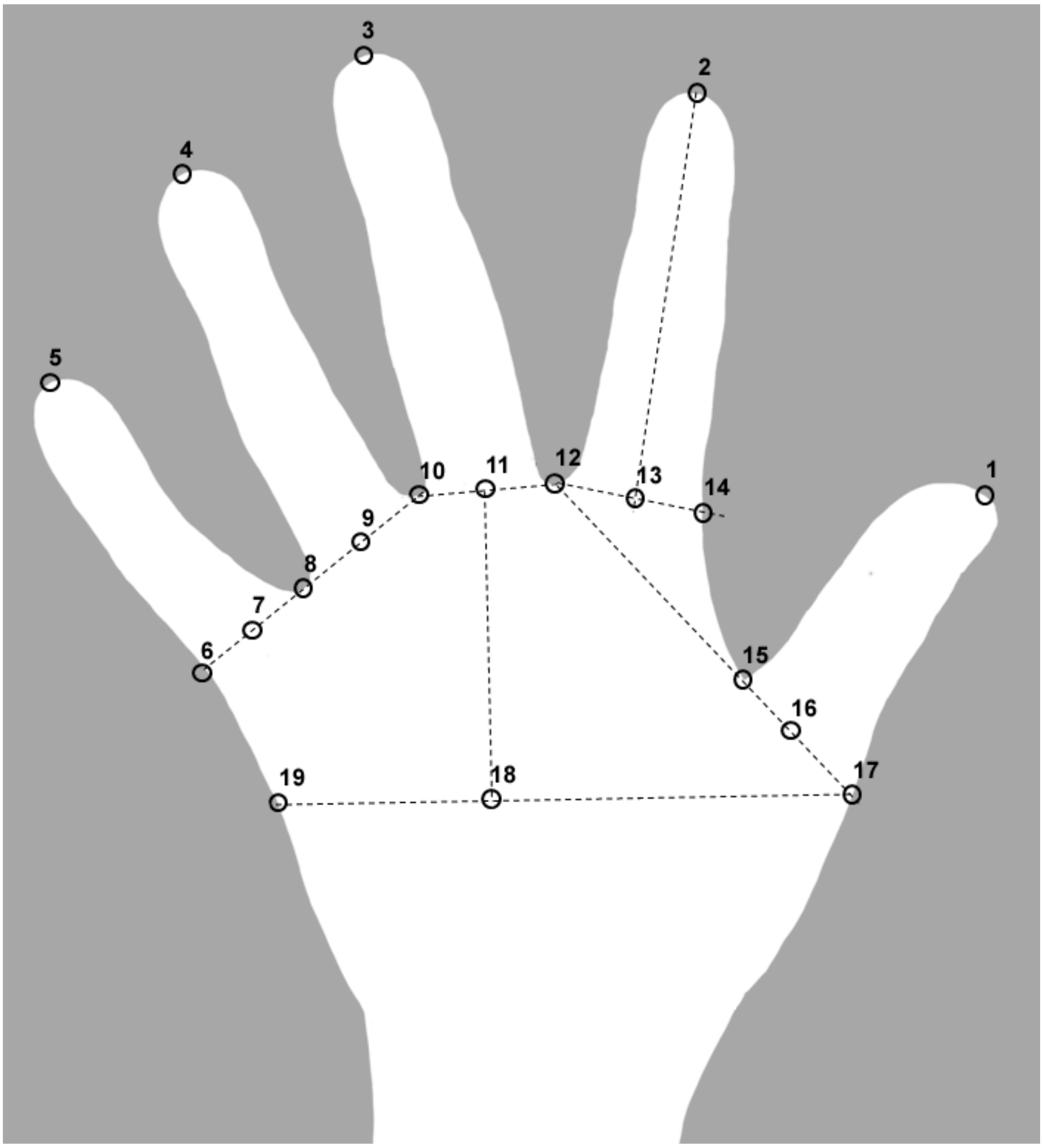


Table 2. Description of the anatomical positions of the 19 Type II and Type II landmarks

Landmark	Type	Anatomical position
1	II	Most distal point of the tip of digit 1 (thumb)
2	II	Most distal point of the tip of digit 2 (index finger)
3	II	Most distal point of the tip of digit 3 (middle finger)
4	II	Most distal point of the tip of digit 4 (ring finger)
5	II	Most distal point of the tip of digit 5 (little finger)
6	III	The proximo-medial point of digit 5 on the medial edge of the hand at the intersection with the palmer digital crease. Established by a line straight line passing between the lowest point of the curve at the base of the fingers (between digits 3 and 4 and 4 and 5) out to medial edge of the hand
7	III	A point on the palmer digital crease at the base of digit 5 (little finger) that is centrally placed, in the midline, directly opposite to landmark 5
8	III	The most superior point of the curvature (webbing) between digits 4 and 5.
9	III	A point on the palmer digital crease at the base of digit 4 (ring finger) that is centrally placed, in the midline, directly opposite to landmark 4
10	III	The most superior point of the curvature (webbing) between digits 3 and 4
11	III	A point on the palmer digital crease at the base of digit 3 (middle finger) that is centrally placed, in the midline, directly opposite to landmark 3
12	III	The most superior point of the curvature (webbing) between digits 2 and 3
13	III	A point on the palmer digital crease at the base of digit 2 (index finger) that is centrally placed, in the midline, directly opposite to landmark 2
14	III	The most proximal point of digit 2 on the lateral edge of the hand at the intersection with the palmer digital crease. Established by a line straight line passing between the lowest point of the curve at the base of the fingers (between digits 3 and 2) out to medial edge of the hand which is perpendicular to midline passing between landmark 2 and 13
15	III	The most superior point of the curvature (webbing) between digits 1 and 2
16	III	A point at the base of digit 1 (thumb) that is centrally placed, in the midline, directly opposite to landmark 1. The landmark is situated on a straight line passing from landmark 12, through landmark 15 out to the lateral edge of the hand.
17	III	The point where a straight line passing from landmark 12, through landmark 15 exits the lateral edge of the hand
18	III	A point on the central palm, directly opposite landmark 11, in the midline of the hand. Perpendicular to a line passing across the palm from landmark 17 to the medial edge of the hand
19	III	A point on the medial edge of the hand directly opposite landmark 17 in the transverse plane

4.4. Geometric Morphometric Analysis

The resulting two-dimensional coordinate configurations (*n* 264) were subjected to a generalised Procrustes analysis (GPA) with full-tangent space projection; this was undertaken using the *MorphoJ* package, with additional statistical analyses undertaken using *IBM SPSS v20.* Procrustes superimposition (GPA) ensures that sized-based effects are removed and only shaped-based differences remain; the influence of size on shape can subsequently be investigated by analysing the correlation between the extracted size residual (configuration centroid size) and the remaining shape residuals (in this study we use principal component loadings, although Procrustes coordinates can equally be applied). The following post-hoc tests were applied:

(i) Following GPA the configurations were subjected to a series of principal component analyses (PCA) to explore the relationships between patterns of sexual dimorphism between male and female hands. In this study the shape differences revealed by PCA are visualised by using outline plots between male and female mean shape configurations.

(ii) The utility of the resulting shape variables as an aid in the assessment of biological sex was undertaken using Fisher's linear discriminant analysis based on the residual shape variables. Shape variables were converted into principal component scores, which helps reduce the dimensionality of the data by analysing a limited number of PC scores from the cases instead of the original data; thus only relatively large group mean differences will be represented by the retained lower order PCs, leaving a proportion of the variance unaccounted for. Classification using Fisher's linear discriminant analysis (LDA) based on *p* PC scores (where *p* is the number of PCs retained following step-wise entry); Leave-one-out cross validation was applied to assess performance of the classification. Significance of sexual dimorphism in shape was assessed by Procrustes ANOVA on group means (Klingenberg et al., 2002).

(iii) The following GPA classifications were based on three iterations to separate the effects of size from shape: (1) size-only using log of configuration centroid (centroid size can be used as a biologically meaningful expression of the overall scale of the landmark configuration, and thus of the relative sizes of individual configurations); (2) size-free using *p* PC scores with PC1 excluded (the first principal component is generally considered to carry a residual size-based component); and (3) analysis of form using centroid size, and *p* PC scores including PC1.

(iv) Partial Least Squares analysis (PLS) was performed in MorphoJ (Klingenberg, 2008) within a single configuration to test for structural modularity (Klingenberg and Zaklan, 2000).PLS examines covariation between two or more sets of variables, and identifies features of shape that most strongly covary between blocks; this technique is increasingly being used for studying patterns of integration of parts within single configurations of landmarks thus allowing for an assessment of anatomical or structural modularity. In the present study we use an assessment of modularity to investigate whether anatomical regions (specifically the digital rays and the palmar surface) provide better assessment of biological sex if treated as isolated structural units, or if they improve sex assessment when combined into a single anatomical module.

(v) Stepwise re-sampling and re-analysis of dataset was applied following PLS to optimise shape classification criteria, with size-based, size-free and form-based analyses reapplied to anatomical modular blocks.

(vi) Measurement error in landmark acquisition was assessed by digitising five individual configurations five times on five separate occasions, and then subjecting them to GPA and PCA. Measurement error was assessed visually using the method of O'Higgins and Jones (1998) by comparing variation within the repeat runs against the total configuration sample. Procrustes ANOVA based on Procrustes distance was subsequently used to assess the relative magnitude of error from repeat measurements.

5. Results

5.1. Sample

Participant ages ranged between 17 and 70 years (SD=11.86). There were no significant differences between male age (mean age=33.11, SD=12.07) and female age (mean age=28.92, SD=11.52); ($F_{1,130}$=4.04, p=0.05). Height ranged between 152 and 195cm (SD=9.48). Males were significantly taller (mean height=176.84 cm; SD=8.53) than females (mean height=165.52; SD=7.13); $F_{1,130}$=68.53, p<0.001.

5.2. Stencil analysis – shape and form

5.2.1. Assessment of measurement error

Error testing of repeated runs indicated no significant difference (p=0.884) and therefore low measurement error. The repeat specimens clustered closely together on the principal axes relative to the variation between individuals, suggesting measurement error was small and the dispersal isotropic in nature.

5.2.2. Principal Components Analysis (PCA)

Principal components analysis of the total sample yielded 34 principal components with non-zero variability (2*k*-4 shape variables, where *k* is the number of landmarks). The first three principal components together accounted for 66.98% of variation, with the first ten factors accounting for around 95% of shape variation in the sample (Table 3). Procrustes ANOVA of shape residuals indicated significant size and shape difference between the sexes (centroid only $F=165$, $p< 0.0001$; shape $F=5.27$, $p< 0.0001$). Global shape variation is expressed in Fig. 3 by the visualisation of principal components 1 and 2; the wire-frames indicate sex-based differences. Shape variation within PC1 (which explains 29.75% of total variation) was dominated by relatively shorter palms in females, in conjunction with medial displacement of the root and tip of the thumb (Landmarks[LM] 1, 15 and 17), and lateral displacement of the fifth phalanx (LM 5 and 6) – the residual size component of PC1 confirming that males have relatively larger and broader palms than females are expected. PC2 on the other hand (24.28% of variation) was dominated by relative narrowing and lengthening of the palm in females when corrected for size, and with concomitant narrowing of the phalangeal rays, compared to the male mean shape, the overall shape being more slender.

Table 3. First ten principal component loadings showing eigenvalues and percentage of variation expressed

PC	Eigenvalue	% Variance	Cum. % Variance
1	0.0018435	29.75	29.75
2	0.00150459	24.28	54.03
3	0.00080262	12.95	66.98
4	0.00045589	7.36	74.34
5	0.00034764	5.61	79.95
6	0.00025635	4.14	84.08
7	0.00022347	3.61	87.69
8	0.00017236	2.78	90.47
9	0.00014614	2.36	92.83
10	0.00009352	1.51	94.34

5.2.3. Classification of overall hand morphology based on size, shape and then form

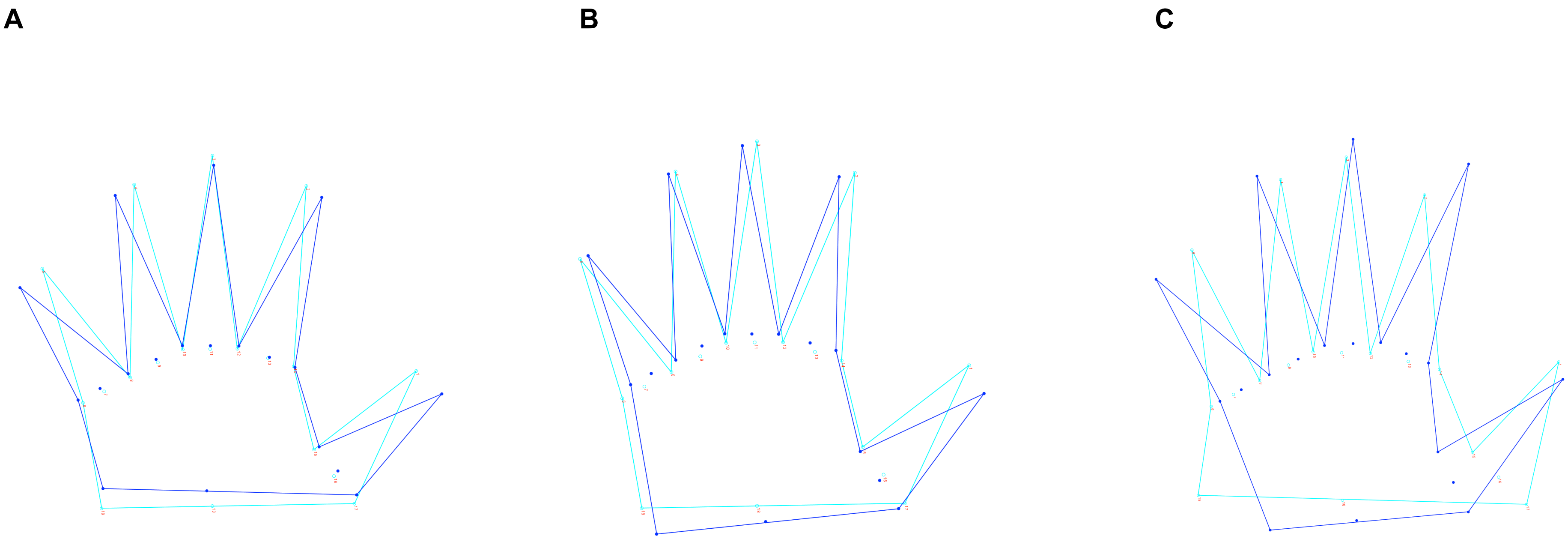
The utility of the resulting shape variables as an aid in assessment of sex was undertaken using Fisher's linear discrimination (DFA) based on PC scores (analyses undertaken with and without size included), with reliability of classification based on leave-one-out validation with permutation test for significance. Three iterations were undertaken: DFA using centroid only, DFA using *p* PC scores (PCs 3, 5 and 7 were retained following step-wise inclusion) to assess using shape, and DFA using size and shape variables (Log of configuration centroids, and *p* PC scores [PCs 1, 2, 3, 5, 7 and 8). The results are displayed in Table 4, including the accuracy of classification divided by sex.

Table 4. Cross-validation results based on complete and re-sampled landmark sets indicating classification based on (centroid) size only, shape only and shape plus size (PC scores and log of centroid size). Table indicates optimal re-sampling results only.

	Predictions utilising size			Predictions utilising shape			Predictions utilising form (size & shape)		
	♂	♀	♂♀	♂	♀	♂♀	♂	♀	♂♀
All 19 - landmarks	76.4	86.1	82.6%	58.5	81.0	72.0%	87.7	91.8	90.2%
Digits 10 - landmarks	76.4	86.1	82.2%	55.7	77.2	68.6%	80.2	90.5	88.4%
Palm - 9 landmarks	76.4	86.1	82.2%	53.8	82.3	70.8%	87.7	93.7	91.3%

Cross-validation of the results indicated that 82.6% of hand shape cases were correctly classified with respect to sex using size alone, with a reduced accuracy when size-based proxies were removed from the discrimination (72.0%). Greatest accuracy was achieved using analysis of form (size and shape) with 90.2% of cases correctly classified. In general females were correctly classified in more cases than males across all three DFA iterations, achieving a maximum accuracy of 91.8% in DFA based on form. A purely shape-based DFA was notably unsuccessful at predicting males based on shape alone, with a classification accuracy of 58.5% being little better than random. The principal discriminating axis of male and female variation displays a similar pattern of shape variation as the first Principal Component (PC1) from the global (total population) sample (see Fig. 3c), with the specific pattern of dimorphism primarily expressed through lower order shape variables; in particular male hands are differentiated from female hands through palm width relative to finger length. This is expressed as relative disto-lateral displacement of the tip of the thumb (Landmark [LM] 1), with supero-lateral placement of the base, and relatively narrower and longer, more slender palms in females.

Figure 3. Visualisation of shape variation for principal components 1 (Fig 3A) and 2 (Fig 3B) for full landmark set (k 19). The wire frames represent the consensus (mean) shape for each sex, with females in dark blue, and males in cyan. Fig 3C shows the sex-based differences between the two consensus shapes following discrimination function analysis.



5.2.5. Partial Least Squares (PLS) analysis to test for structural modularity

Following DFA a 2-block PLS was applied. PLS examines covariation between two or more sets of variables, and identifies features of shape that most strongly covary between blocks allowing for an assessment of modularity. Landmarks were sub-divided between digital (LM 1-5, 7, 9, 11, 13 & 16) and palmar landmarks (LM 6, 8, 10, 12, 14, 15, 17-19). PLS produced an RV coefficient (the measure of covariance) of 0.361; an RV of 1 implies that one set of variables can be obtained from the other set by rigid rotation and/or reflection. An RV of 0.361 indicates a poor degree of association between blocks (implying the variables are largely uncorrelated), and hence a low degree of modularity and morphological integration between the morphology of the digits and the palm.

5.2.6. Stepwise re-sampling and re-analysis of dataset to optimise classification using shape then form

To investigate this further, we performed a series of stepwise exclusion tests removing 10% of landmarks at each stage. Reanalysed data was based on iterations of dependent (both blocks) and independent units (single blocks). This produced an optimum classification based on $k = 10$ landmarks of the digits and $k = 9$ landmarks of the palm. Reanalysis (PCA and DFA) of palm and digits showed that the digital landmarks performed poorly based on shape (68.6%), while the palmer landmarks performed almost as well as the original 19 landmarks (Table 4). Incorporating size – to capture form - improved these scores, and enabled 91.3% of our sample to be assigned to the correct sex category (Table 4). Stepwise permutation tests and analyses of regional covariation indicate a lack of functional integration in the structure of the hand, with a low degree of anatomical modularity between the digital rays and the palm suggesting that functional ties between the units do not necessarily covary in influencing sex-based morphological expression. Consequently such units can be studied either together or independently; the latter is important as it allows for relatively accurate assessment of sex based purely on the palm alone – thus allowing sex assessment in cases where digits are either missing, or imprecisely rendered.

6. Discussion

We have argued above that, in the first instance, it makes more sense to attempt to describe the context in which particular hand image sets were made by identifying the age and sex of the individuals present through their hand images. This is essential, since, despite the common occurrence of hand images dated to the Palaeolithic across many parts of the world, the considerable diversity of meanings assigned to these images in ethnographic accounts, and the variety of contexts in which such images were made, renders it impossible to offer any type of meaning based on the nature of the images themselves. Of these two attributes - age and sex - the determination of sex must be primary, since determination of age often involves a judgement based on relative size amongst prints of the same sex. For studies of prehistoric cave art, the importance of a correct determination of sex to an understanding of the human context of hand image making is considerable. We must therefore persist in examining new methods for the determination of the sex of these images and not simply give up the challenge as a lost cause.

As noted above, previous attempts to identify the sex of the individuals whose hands were stenciled in the Palaeolithic have mainly use variation in hand size (eg. Guthrie, 2005; Snow, 2006, 2013) and/or digit length ratios of contemporary samples (eg. Snow, 2006, 2013; Table 1). Using hand size data, however, is highly problematic because there is such a large degree of overlap within any single population between the sizes of male and female adult hands and between the sizes of male and female sub-adults and adult hands. As a consequence of this overlap in size, authors have argued that it is impossible to effectively distinguish the sex of Palaeolithic hand prints or stencils except for those limited number of the smallest or largest hands (Guthrie, 2005; Galeta et al., 2014). In actuality, it is only the very largest adult hands that a sex judgment can be made with any confidence, because the smallest adult hands overlap in size with adolescent individuals (Guthrie, 2005). Similar problems exist when using digit length data (i.e.2D:4D), where there is known to be both a considerable overlap in digit ratios between males and females within a population (~60%; McIntyre, 2006), and between ethnically different populations leading some authors to suggest that digit ratios should only be used where the parameters of the population under study are known (Nelson et al., 2006).

In this paper we have presented a new method of predicting the sex of makers of hand stencils that is not based on hand size alone, but also uses shape, which can then be qualified by size if required. In particular, we have aimed to present a methodologically and statistically robust means of comparing sex-based hand shape using geometric morphometric methods. This study has demonstrated that geometric classifier variables have utility in assessing biological sex from hand shape; our results show that this method results in a successful assessment of the sex of our reference sample of hand stencils of between 68.6 and 93.7% depending on which anatomical region is sampled (Table 4). It is clear that different signals are presented indicating this dimorphism is either shape-based or sized-base, and a combination of the two (form-based). Patterns of sexual dimorphism elsewhere in the human body are often expressed through size-based differences between males and females. On the basis of this study the hand appears to follow this format, with sized-based dimorphism clearly present in the current sample as expressed by centroid size; mean male centroid size for the hand overall was 3282.3 and the female mean 3013.3 and these provided a biologically meaningful expression of the overall scale of landmark configuration. As such, the differences between male and female centroid size was found to be highly significant (ANOVA F=165.8, p<0.0001), with males having generally larger hands than females; clearly size plays an important part in assessment of biological sex from the hand, though as the summary of results in table 4 indicate, the degree of classification accuracy is generally less effective using centroid as a size-proxy alone, than when it is combined with shape variables.

Whilst multivariate regressions of the retained shape variables from discriminant analysis against sex indicated a significant degree of sexual dimorphism in the total sample with respect to shape, shape variables and centroid size were found to be largely uncorrelated in the global sample. The correlation coefficient for both sexes regressed to centroid size was only 0.446 (R^2 = 0.199, F=6.294, p<0.0001), with the separated R^2 coefficients for males and females of 0.353 and 0.397 respectively. Thus, regression of shape variables against centroid size indicated that male and female hands present significant shape-based and size-based independent effects, but that these differences are not correlated with any observable allometric trajectory. Only limited correlation was observed between extracted size and residual shape variables suggesting that size does not play a significant part in influencing hand shape between the sexes; thus, males have 'male shaped' hands and females have 'female shaped' hands regardless of the overall size of the individual, but that taken together with the significant differences between male and female centroid size, a sex-based interplay between the two is recognised. The recognition of a sex-specific shape pattern is broadly in keeping with current understandings of the effect of high prenatal levels of testosterone upon the ratio between the second and fourth digits, where a low 2D:4D ratio is generally seen in males. It is now understood that prenatal sex hormones regulate the plethora of genes that control the proliferation of chondrocytes that lead to sexual differences in the growth of the digits (Zheng and Cohn, 2011). It is apparent that the fourth digit is particularly sensitive to the process of prenatal androgen effects (PAE) leading to longer lengths in the fourth digit of those individuals' subject to a high PAE, with sex-specific 2D:4D ratios evident from as early as nine weeks intrauterine suggesting that the human hand represents a recognisably sexually-dimorphic region from a very early age (Nelson et al. 2006).

Our results closely mirror those of Sanfilipo et al., (2013), who, using GMM analysis of hand shape based on digital scans, showed that females were correctly classified to the same extent using either shape or size (81.4%), while centroid size was an even more effective predictor for males (80.7%). Importantly, we have shown that this rate of successful sex determination can be achieved using (indirect hand) data derived from an analysis of stencilled images with all their attendant variation in size, lack of many soft-tissue anatomical landmarks (e.g., finger creases) and potential distortions due to the direction of paint spray, for example.

In addition to the high success rate of our method for sex determination, our method also significantly advances this field of study because it addresses one of the key problems encountered in the examination of Palaeolithic-age hand images: the completeness, or lack of, of many of the stencilled images. This is an important step forward because, in reality, most hand stencils are incomplete; the finger tips in particular are the region in which the image is lost or resolution is poor (Flood, 1987; Snow, 2013). Chazine and Noury (2006), for example, could only apply their method to 34 hands from Gua Masri II, part of the Kalimantan Caves in Borneo that contain many hand stencils (Chazine, 2005). Snow, in his most recent and considerably expanded study (Snow, 2013), was only able to use 32 hand stencils after close scrutiny of hundreds from the caves of Spain and France. Furthermore, in some of the Palaeolithic sites with the largest numbers of hand images, such as Gargas, Chauvet or Cosquer, digit data is entirely missing possibly due to a deliberate manipulation of the hand in stencilling or printing, and the base of the palm often appears unclear probably due to the difficulties of making an effective image of the hand in this area when stencilling.

The most significant advantage in the use of GMM analysis based on the suite of landmarks we have described above is that the number of Palaeolithic images that can be examined increases without major detriment to the quality of the results. In our study, the analysis of shape based on palm landmarks performed better than the digit landmarks in all analyses (Table 4). This concurs with other studies that have indicated that the shape of the palm is more sexually dimorphic than other regions of the hand when examining scans (Ishak et al., 2012; Kanchan and Rastogi, 2009; Sanfilipo et al., 2013). Our results confirm that, for the analysis of data from hand stencils, the palm is a more suitable region when compared to the fingers for the determination of sex. We can suggest, therefore, that as long as the palm-area on hand stencils is complete, the accuracy of predicting sex correctly should be high. The suitability of data derived from palm images for sexing the makers of hand stencils should enable a significant increase in numbers of hand stencils that can be analysed.

It is important to emphasise, however, that whilst the results presented here indicate that there might be an effective way to assess the sex of hand images based on shape, this method still needs to be tested on hand images collected from a range of human populations. Like other earlier studies we have based our examination on a reference sample of stencils made using the hands of adults from an almost exclusively northern European background, as was the case in earlier studies by Snow (2006, 2013) and Guthrie (2005). But in cases in which Palaeolithic images are analysed, the use of an appropriate reference sample is essential since both hand size and digit ratio (2D:4D) vary between different ethnic populations (Galeta et al., 2014; Manning, 2002; Manning et al., 2007). The need for an appropriate reference sample has been effectively demonstrated by Snow (2013) who tested his algorithm (based on a sample of North American individuals each with northern European-ancestry) on a sample of handprints of Native Americans of known sex, with disappointing results. In another test, Galeta and co-workers (Galeta et al., 2014) applied Snow's algorithm to data derived from hand scans from a contemporary French reference sample, and found that contemporary French males were more likely to be identified as female using Snow's method.

The earlier studies by Snow (2006, 2013), Guthrie (2005) and Groenen (1988) have either explicitly argued, or assumed, that methods derived from hand scans of individuals of northern European ancestry are an appropriate reference sample (for European Palaeolithic-age hand stencillers) because northern Europeans are the descendant population of those individuals whose hands were imaged at Palaeolithic sites in Western Europe. In support of this judgement, Snow (2013) cites the conserved nature of the Y chromosome in European populations suggesting continuity since the Upper Palaeolithic (Semino et al., 2000). Other studies based on contemporary human DNA have also suggested that northern Europeans can primarily trace their ancestry to the human populations that recolonised Europe from a southwestern refuge following the Last Glacial Maximum (Pereira et al., 2005; Torroni et al., 2001).

Unfortunately, this assumption cannot be so easily retained in the light of the more recent studies that benefit from the addition of data on ancient DNA extracted from skeletal material of Palaeolithic and Neolithic age and new techniques for gene sequencing and modelling the genetic history of hominin populations (see Barbujani, 2012; Bramanti et al., 2009; Brandt et al., 2015; Lacan et al., 2013; Lazaridis et al., 2014; Pala et al., 2012; Pinhasi et al., 2012; de-la-Rua et al., 2015; Soares et al., 2010; amongst others). Specifically, these studies now raise potential problems in the earlier use of genetic data to argue that a contemporary European sample is the best analogue for those pre-Last Glacial Maximum populations that produced hand stencils on the basis of an assumption that there is a simple, direct and complete continuity between human populations living in Europe before the Last Glacial Maximum to modern European populations. The first problem relates to the potential addition of new lineages to the surviving Last Glacial Maximum populations of Europe through the influx and admixture of new people from populations that survived in diverse refuge areas during the Last Glacial Maximum, and later again from new populations arriving into Europe alongside the spread of agriculture into the continent. The second problem relates to the potential loss of certain European lineages during the extreme environmental conditions that occurred in Europe during the Last Glacial Maximum. Since the large majority of Palaeolithic age hand stencils and prints in Europe predate the Last Glacial Maximum it is the continuity, or not, of these populations through to the modern European population that is at issue, and not the homogeneity of modern European populations.

For example, research by Soares and others has suggested that modern European populations are not simply the outcome of a major hominin recolonization of Europe from a Franco-Cantabrian refuge area; there is also evidence for admixture from groups expanding out of other refugia located in Italy, the Balkans and possibly Eastern Europe (Soares et al., 2010), and this has since been expanded to include the influx of new populations from the Near East (Pala et al., 2012). Even within modern Franco-Cantabria there appears to be evidence for genetic variability (de-la-Rua et al., 2015) resulting from different patterns of hominin expansion after the Last Glacial Maximum. Bramanti et al., (2009) and Skoglund et al., (2012), amongst others, have indicated that there was a genetic discontinuity between the first incoming agricultural populations into Europe and pre-existing local indigenous populations, and that this was followed by a process of replacement and/or admixture between the two. Ancient DNA extracted from two Upper Palaeolithic humans dating from before the Last Glacial Maximum reveals a much greater variability in their genetic make up when compared to Mesolithic age humans (Raghavan et al., 2014; Seguin-Orlando et al., 2014). Whilst this data comes from burial sites geographically located in the Ukraine (Kostenki) and central Siberia (Mal'ta), these individuals are genetically related to Western Eurasian populations.

Furthermore, changes in social structure associated with agriculture might have altered the hormonal profiles of these populations leading to changes in hand morphology (Cieri et al., 2014), and this is particularly pertinent for methodologies that incorporate finger lengths and digit ratios (e.g., Snow, 2006, 2013), because the difference between digit lengths as measured by 2D:4D analyses relies upon such differences being the result of variation in prenatal hormonal differences (Manning et al., 1998; Nelson et al., 2006). The high degree of sexual dimorphism in hand size between males and females recognized by Snow (2013) also suggests that there may be significant differences in social structure, and hence prenatal hormones, between modern Europeans and the Palaeolithic human populations that made the hand images.

7. Conclusion

In conclusion, we have presented here a new method for recording and determining the sex of individuals from their hand stencils. This method employs a GMM analysis of shape based on a suite of clearly identifiable landmark points on the hand. Results suggest that there is a significant difference between the shape of male and female hands that permits a high degree of accuracy in determining their sex. Furthermore, our method also allows us to assess the sex of hand images where digits may be missing or shortened because of the importance of palmar region in determining a difference in shape between male and female hands. This method represents a significant improvement on previous studies that have relied primarily on differences in hand size and digit ratios that are known to overlap greatly between males and females, and also offers the potential for application to a much greater number of images than currently examined. The method still needs to be tested on different reference samples from contemporary populations that are not of northern European descent, and this will be the focus of future work by this research team.

Finally, in our review of the previous studies of Palaeolithic hand images we noted that it was impossible to compare across studies because the dimensions taken from hands were not consistent across studies and the size data were unavailable for re-analysis. We therefore encourage other researches to use the protocol and landmarks outlined in this study to begin to analyse hand stencils from other ethnic groups. For this field of research to progress, we must begin to standardise methods and collaborate across research groups with the aim of developing a robust, predictive methodology based on diverse modern samples before it is applied to Upper Palaeolithic hand stencils.

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