Nonhuman Primate Hybridization and the Taxonomic Status of Neanderthals

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ABSTRACT The present study examines the taxonomic status of Middle Paleolithic Neanderthals by comparing their observed minimum genetic divergence from Upper Paleolithic modern humans in Europe with that observed between macaque species from Sulawesi that are known to hybridize and fully intergrade in the wild. The genetic divergence, and differentiation between Neanderthals and Upper Paleolithic modern humans, as indicated by pairwise minimum genetic distances and F_{ST} values calculated from the estimated minimum genetic relationship (R) matrix derived from craniometric data, are sig-

nificantly greater than those observed both between hybridizing and noninterbreeding Sulawesi macaque species, suggesting that mate recognition and the possibility of gene flow between Neanderthals and Upper Paleolithic modern humans might have been greatly reduced. These results support a species-level taxonomic distinction for the Neanderthals as suggested by proponents of the replacement model. Furthermore, assumptions regarding the monophyletic origin of modern humans from outside Europe are likely valid. *Am J Phys Anthropol* 115:157–166, 2001. © 2001 Wiley-Liss, Inc.

The origin of anatomically modern humans has been the subject of considerable recent debate in the anthropological literature (for overview, see Frayer et al., 1993; Smith et al., 1989; Straus, 1995; Stringer, 1992; Wolpoff, 1992). Much of this debate centers on the various interpretations of morphological (i.e., genetic) affinity among the Middle and Upper Paleolithic hominid fossils, and the origin(s) of anatomically modern humans in Europe. Determining to what extent, if any, the Middle Paleolithic Neanderthals of Europe contributed to the Upper Paleolithic modern human gene pool has been a subject of recent investigation by Turbón et al. (1997). The answer to this particular question has obvious taxonomic and phylogenetic implications. Using a species definition fostered by the biological species concept, any gene flow between Middle Paleolithic Neanderthals (*Homo neanderthalensis*) and anatomically modern humans (*Homo sapiens*) would imply they were conspecific. Conversely, the absence of measurable gene flow implies a species-level distinction for the Neanderthals, allowing for a monophyletic origin for anatomically modern humans outside of Europe.

Briefly, the participants in this debate typically lend support to one of three models: 1) the multiregional model, 2) the replacement model, and 3) an admixture model. The multiregional model assumes phyletic gradualism from archaic *Homo sapiens* to anatomically modern humans, with continuing gene flow between Neanderthals and Upper Paleolithic modern human populations likely (e.g., Frayer et al., 1993; Wolpoff, 1989; Wolpoff et al., 1984). The re-

placement model, however, requires a relatively abrupt displacement of regional archaic human populations in Europe and Asia by morphologically, culturally, and behaviorally distinct anatomically modern humans, presumably from Africa (Stringer and Andrews, 1988; Stringer and Gamble, 1993). Hypotheses regarding mate recognition and gene flow between Middle Paleolithic Neanderthals and Upper Paleolithic modern humans are typically rejected by supporters of the replacement model (Cann, 1987, 1988; Cann et al., 1987). The third model allows for the possibility of limited admixture, or hybridization, between Neanderthal populations and modern humans dispersing into Europe (cf. Bräuer, 1984; Duarte et al., 1999; Simmons, 1994, 1999; Smith and Trinkaus, 1991).

The debate fostered by the replacement and multiregional models has incorporated archaeological (d'Errico et al., 1998; Hublin et al., 1996; Klein, 1992; Mellars, 1989), morphological (Pearson, 2000), molecular (Krings et al., 1997, 1999; Ovchinnikov et al., 2000; Scholz et al., 2000), and quantitative genetic (Relethford, 1998; Relethford and Harpending,

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1994) perspectives. Results presented by recent molecular and morphometric studies seem to indicate that Neanderthals were genetically distinct from anatomically modern humans, and that gene flow between these congeners was curtailed (Krings et al., 1997, 1999; Ovchinnikov et al., 2000; Scholz et al., 2000; Turbón et al., 1997; but see Norborg, 1998).

MORPHOMETRIC DATA AND BIOLOGICAL DISTANCE

The use of morphometric data to assess genetic relationships among human and nonhuman primate populations is common within biological anthropology. Although measurement values for metric traits are a product of both hereditary and environmental factors, research presented in the anthropological literature concerning human quantitative genetics indicates a moderate degree of genetic control for many head and face measurements (Hiernaux, 1963; McHenry and Giles, 1971; Osborne and DeGeorge, 1959; Devor, 1987; Susanne, 1977). Susanne (1977), for example, presents narrow-sense heritability values (h^2) ranging between 0.391–0.715 for 19 head and face measurements, based on a study of Belgian families. Devor (1987) reports an average narrow-sense heritability of 0.55 for head and face measurements. This relatively high level of heritability demonstrates the utility of craniometric variables in genetic distance studies.

Previous research

Multivariate and quantitative genetic analyses of morphometric data are increasingly being used in human paleontology to investigate the origins of modern humans and to estimate historical relationships among Pleistocene hominids (e.g., Donnelly et al., 1998; Kidder, 1999; Kidder et al., 1992; Relethford and Harpending, 1994; Turbón et al., 1997). Recently, Turbón et al. (1997) claimed a monophyletic origin for modern humans in Europe, "independent of the Neanderthals, whose morphological traits in the face are clearly distinct from those modern human populations analyzed," based on a discriminant analyses of Lower, Middle, and Upper Paleolithic fossils, as well as Iberian Mesolithic and recent modern human samples. This study was based on a discriminant analysis of the first three principal components calculated from a covariance matrix derived from the measurement values of 25 craniofacial variables. Because the raw data were neither size-corrected nor standardized, the resultant principal component scores represent size-shape variables. The authors then estimated historical relationships using a cluster analysis based on Mahalanobis D^2 values derived from the linear discriminant functions. Based on the results of these multivariate analyses, the authors presented an argument for the morphological distinctiveness of Middle Paleolithic Neanderthals in relation to Up-

per Paleolithic modern humans from Europe and recent modern human samples. The divergence of Neanderthals, the authors argued, supports the replacement model by indicating a monophyletic origin for Upper Pleistocene modern humans in Europe, independent (genetically) of Neanderthals. Although Turbón et al. (1997) presented a cogent argument for the distinctiveness of the Neanderthals, what is unclear from their discussion is how morphological distinctiveness is deemed taxonomically significant. Moreover, how much observed morphological divergence is needed to demonstrate relative genetic isolation between Neanderthals and Upper Paleolithic modern humans?

Questions such as these regarding morphology and genetic isolation in the human and nonhuman primate fossil record should be addressed through empirical study of reproduction and morphology among extant primate taxa (see Prat, 2000a,b; Simmons, 1994, 1999). Determining what level of morphological divergence corresponds to genetic isolation among hominid fossils can be accomplished by evaluating the level of divergence between morphologically distinct extant primate species with, and without, known hybrids. The anthropological literature is replete with reports of natural hybridization occurring between morphologically and genetically distinct primate taxa, such as the reported hybridization between *Papio hamadryas anubis* and *P. h. hamadryas* (Phillips-Conroy and Jolly, 1986; Phillips-Conroy et al., 1991), between *Hylobates muelleri* and *H. albibarbis* (Marshall and Sugardjito, 1986), between *H. lar* and *H. pileatus* (Brockelman and Srikosamatara, 1984), and between karyotypically distinct subspecies of *Lemur fulvus* (Tattersall, 1993).

Hybridization has also been reported among the highly variable Sulawesi macaque taxa (Bynum et al., 1997; Ciani et al., 1989; Froehlich and Supriatna, 1996; Groves, 1980; Watanabe and Matsumura, 1991; Watanabe et al., 1991a,b). The examples of macaque hybridization from Sulawesi are particularly interesting because the morphological variation among the seven macaque taxa endemic to this island (Fig. 1) equals or exceeds that of all other non-Sulawesi macaque species according to Albrecht (1978). Despite significant differences in character traits such as pelage patterning, size and shape of the gluteal fields, female sexual swelling, body size, and various facial measurements (Bynum et al., 1997; Froehlich and Supriatna, 1996; Froehlich et al., 1999; Supriatna, 1991), all of which are likely important in maintaining specific mate recognition systems (SMRS) (Paterson, 1985), hybridization occurs across at least four of the seven grossly defined geographic species boundaries on Sulawesi.

Hybridization and phenotypic divergence

The degree of phenotypic divergence observed between the parental taxa of known hybrids can be used as a relative measure for assessing interpopu-

Fig. 1. Map of Sulawesi, Indonesia, showing general locations of the seven macaque taxa endemic to the island, including the *M. tonkeana*-*M. maurus* hybrid (Hybrid).

TABLE 1. Paleolithic fossil specimens included in the multivariate analysis presented by Turbón et al. (1997)

Lower Paleolithic	Middle Paleolithic	Qafzeh	Upper Paleolithic
Bodo	La Chapelle	Qafzeh 6	Mladeč 1
Broken Hill	Forbe's Quarry	Qafzeh 9	Dolní Věstonice 3
Petralona 1	La Ferrassie 1		Předmostí 3
Arago 21	Guattari 1		Předmostí 2
Saccopastore 1	Saint Césaire		Cro-Magnon 1
Saccopastore 2	Shanidar 1		Cro-Magnon 2
	Shanidar 5		Grotte des Enfants 6
	Amud 1		Abri Pataud 2

lational phenotypic variability, or as a comparative threshold for assessing taxonomic significance. For example, if Neanderthals were taxonomically distinct at the species level from Upper Paleolithic modern humans, with no interspecific gene flow, then we would expect the degree of phenotypic divergence to exceed the observed divergence between primate parental species with common hybrids.

The quantitative analysis of phenotypic divergence among Lower Paleolithic archaic humans, Middle Paleolithic Neanderthals, and Upper Paleolithic modern human fossils (Table 1) presented by Turbón et al. (1997) was conducted with the purpose of determining whether these groups can be discriminated, and to establish their variability in relation to recent human samples. Although not explicitly, the authors imply that the morphological divergence

TABLE 2. Sulawesi macaque sample information

Taxon	Sample size	Estimated number of subgroups
<i>Macaca tonkeana</i>	12	3
<i>Macaca maurus</i>	24	2
<i>Macaca nigra</i>	13	2
<i>M. tonkeana</i> - <i>M. maurus</i> hybrid	31	3

TABLE 3. Craniometric variables for Pleistocene hominid samples¹

1. XFB Maximum frontal breadth
2. NLH Nasal height
3. OBB Orbital breadth
4. MAB Palate breadth
5. ZMB Bimaxillary breadth
6. NPH Nasion prosthion height
7. OBH Orbital height
8. NLB Nasal breadth
9. MDH Mastoid height
10. SSS Bimaxillary subtense

¹ Measurements taken in accordance with methods described in Howells (1973, 1989); see Turbón et al. (1997).

demonstrated by their results represents strong evidence of genetic isolation between Neanderthals and Upper Paleolithic modern humans. While the results presented by Turbón et al. (1997) indicate a high degree of morphological divergence, the presumed genetic isolation between Neanderthals and Upper Paleolithic modern humans was not established.

The present study compares the degree of minimum genetic divergence and differentiation between Neanderthals and Upper Paleolithic modern humans with that observed between Sulawesi macaque taxa that hybridize naturally, as well as between species that do not (Table 2). The hypothesis that Middle Paleolithic Neanderthals were genetically isolated from Upper Paleolithic modern humans without gene flow is tested by comparing their observed minimum genetic divergence and differentiation, as described by minimum genetic distances (d^2) and F_{ST} values, with those values observed between both noninterbreeding and hybridizing Sulawesi macaque taxa. If the hypothesis of genetic isolation is true, we would expect greater minimum genetic distance and F_{ST} values between Middle Paleolithic Neanderthals and Upper Paleolithic modern humans than is seen between naturally hybridizing Sulawesi macaque taxa.

MATERIALS AND METHODS

Samples

Data on 10 craniometric variables for four Paleolithic samples from Europe and the Middle East were kindly provided by Dr. C. Stringer (Table 3). These data are the same used by Turbón et al. (1997) in their analysis of Pleistocene hominid variation. The original data set used by these authors was reduced for the present study by eliminating those

TABLE 4. Head and face measurements for the Sulawesi macaques

1. Maximum cranial length
2. Cranial vault length
3. Upper facial height ¹
4. Biauricular breadth
5. Bizygomatic breadth
6. Bifrontal breadth ²
7. Upper bicanine breadth
8. Lower Bi-P3 breadth

¹ Similar to nasion-prosthion height (NPH).

² Similar to maximum frontal breadth (XFB).

variables based on angles, radii, and fractions, as well as most subtenses. The resultant data set is more comparable to the available Sulawesi macaque data set in both variable composition and number. It is important to note that the variables used by Turbón et al. (1997) with the highest eigenvector loadings were not eliminated (see Turbón et al., 1997, their Table 3a). The present study also uses similar metric data on eight head and face variables (Table 4) taken on wild-caught and live pet macaques from the Indonesian island of Sulawesi between 1991–2000. The Sulawesi macaque data set includes three species: *Macaca tonkeana*, *M. maurus*, and *M. nigra*, as well as one hybrid sample (Table 2). The hybrid sample is the product of full, reciprocal introgression between *M. tonkeana* and *M. maurus* (Supriatna, 1991; Froehlich and Supriatna, 1996). Both the Sulawesi macaque and Paleolithic hominid samples include males and females.

Analyses

Multivariate estimates of the minimum genetic relationship (R) matrix for both the Sulawesi macaque and Paleolithic craniometric data sets were generated using the methods described by Williams-Blangero and Blangero (1990), Relethford and Blangero (1990), Relethford (1994), and Relethford et al. (1997). All of the following quantitative genetic analyses were conducted using RMET, a computer software package provided by Dr. J. Relethford (for detailed description of computations, see Relethford et al., 1997). Graphical representations of the results, including principal coordinate ordination and average linkage cluster analyses, were generated using SYSTAT 9.0 (Wilkinson, 1990). Principal coordinate ordination was used in addition to cluster analysis, because dendrograms generated from these analyses are sometimes dependent on the specific clustering algorithm chosen (Relethford and Harpending, 1994).

The RMET program calculates the minimum R matrix for g groups based on t traits by first standardizing the data using a z-score transformation and then calculating the codivergence matrix using the standardized data (Relethford et al., 1997). When metric traits are used, the elements of the phenotypic codivergence matrix (C) are computed as

$$c_{ij} = (\mathbf{x}_i - \boldsymbol{\mu})' \mathbf{P}^{-1} (\mathbf{x}_j - \boldsymbol{\mu}) / 2t = \Delta_i' \mathbf{P}^{-1} \Delta_j / 2t \quad (1)$$

where $\mathbf{x}_i$ and $\mathbf{x}_j$ are the mean vectors for groups i and j , $\boldsymbol{\mu}$ is a vector of means over all groups, $\mathbf{P}$ is the pooled within-group variance-covariance matrix, Δ_i and Δ_j are g by t matrices consisting of the deviations of group means from the total mean pooled over all groups, and prime (') indicates matrix transposition (Relethford, 1997). Preferably, the pooled within-group variance-covariance matrix, as well as the vector of means over all groups, is weighted by relative population size (w_i) (Relethford and Blangero, 1990). When the relative population sizes are unknown, as is the case with our data sets, RMET allows w_i to be set to 1, resulting in an unscaled R matrix.

The average diagonal value of the C matrix is used to calculate genetic differentiation among groups (F_{ST}), which is the average genetic distance to the centroid over all populations (Relethford and Harpending, 1994), using Equation 2:

$$F_{ST} = c_0 / 1 + c_0 \quad (2)$$

where t is the number of traits and c_0 is the average diagonal value of the C matrix. Relethford and Harpending (1994) showed that the R matrix can then be related to the C matrix as

$$\mathbf{R} = \mathbf{C}(1 - F_{ST}). \quad (3)$$

Because we are interested in comparing the genetic divergence between Neanderthals and Upper Paleolithic modern humans with that observed between morphometrically diverse Sulawesi macaque species that hybridize naturally in the wild, minimum genetic distances (d^2) were derived from the R matrix, using Equation 4:

$$d_{ij}^2 = r_{ii} + r_{jj} - 2r_{ij} \quad (4)$$

where the diagonal elements of the R matrix, r_{ii} , are the genetic distances of population i from the centroid (Relethford and Harpending, 1994; Harpending and Jenkins, 1973).

The hypothesis that the genetic divergence and differentiation of Middle Paleolithic Neanderthals and Upper Paleolithic modern humans are greater than those observed between naturally hybridizing Sulawesi macaque taxa was tested by comparing d^2 and F_{ST} values, using a modified Z-test using pooled standard errors (see Relethford et al., 1997). All d^2 and F_{ST} values used for hypothesis testing were pairwise-calculated, using RMET. Minimum genetic distances and F_{ST} values were also calculated for the combined macaque and Paleolithic samples.

RESULTS

The dendrogram generated from our average linkage cluster analysis of the minimum genetic distances, d^2 , derived from the estimated minimum R matrix for the Sulawesi macaques (Fig. 2) shows *M. tonkeana* grouped closely with its conspecific *M. maurus* hybrid (Hybrid), and secondarily grouped with *Macaca maurus* at an approximate rescaled branching distance of 0.35. Predictably, *M. nigra*, as

Fig. 2. Dendrogram of Sulawesi macaque samples generated from average linkage cluster analysis, using unbiased minimum genetic d^2 distances derived from the estimated minimum R matrix. NIGRA, *M. nigra*; MAURUS, *M. maurus*; TONKEANA, *M. tonkeana*; Hybrid, *M. tonkeana*-*M. maurus* hybrid sample.

Fig. 3. Principal coordinate ordination of the first two eigenvectors of the estimated minimum R matrix, using equal relative population weights ($w_i = 1$). Each eigenvector is scaled by the square root of its corresponding eigenvalue. NORTH and EAST orient the plot to approximate the geographic positions of the Sulawesi macaque taxa (cf. Fig. 1).

the only noninterbreeding taxon included in this study, appears as an outgroup. This same pattern of *M. tonkeana* grouped with its hybrid is shown by the two-dimensional principal coordinate ordination of the first two scaled eigenvectors of the estimated minimum R matrix, comprising 68.9% and 23.6% of the variation (Fig. 3). The intermediate plotted positions of the hybrid, as well as the fact that the principal coordinate ordination corresponds qualitatively with the relative geographic positions of these

Fig. 4. Dendrogram of Paleolithic hominid samples generated from average linkage cluster analysis, using unbiased minimum genetic d^2 distances derived from the estimated minimum R matrix. UP, Upper Paleolithic; MP, Middle Paleolithic; Q, Qafzeh; LP, Lower Paleolithic.

Fig. 5. Principal coordinate ordination of first two eigenvectors of estimated minimum R matrix, using equal relative population weights ($w_i = 1$). Each eigenvector is scaled by the square root of its corresponding eigenvalue.

macaque populations, suggest measurable gene flow across putative species boundaries.

The dendrogram generated from the average linkage cluster analysis of the Paleolithic hominid d^2 values (Fig. 4) agrees with that presented by Turbón et al. (1997, their Fig. 5). The two-dimensional principal coordinate ordination of the first two scaled eigenvectors of the estimated minimum R matrix, comprising 71.1% and 20.4% of the variation (Fig. 5),

TABLE 5. Estimated minimum R matrix derived from the Paleolithic craniometric data set using equal population weights ($w_i = 1$) and an average narrow-sense heritability of 0.55 is presented below the diagonal¹

	LP	Q	MP	UP
LP	0.6421 (0.0969)	0.8192 (0.2275)	0.5822 (0.1237)	3.6266 (0.2791)
Q	-0.0779 (0.0820)	0.0212 (0.0926)	1.0813 (0.2348)	0.9442 (0.2229)
MP	0.2473 (0.0551)	-0.3126 (0.0668)	0.4348 (0.0627)	2.4604 (0.2022)
UP	-0.9115 (0.0731)	0.1193 (0.1011)	-0.4319 (0.0583)	1.1616 (0.0984)

¹ The diagonal represents the unbiased diagonal values of the estimated R matrix. Listing of unbiased minimum genetic distances (d^2) are found above the diagonal. The d^2 values listed were not pairwise-calculated. Standard errors are in parentheses. LP, Lower Paleolithic; MP, Middle Paleolithic; UP, Upper Paleolithic; Q, Qafzeh 6 and 9.

TABLE 6. Estimated minimum R matrix derived from the Sulawesi macaque craniometric data set using equal population weights ($w_i = 1$) and an average narrow-sense heritability of 0.55 is presented below the diagonal¹

	<i>M. tonkeana</i>	<i>M. maurus</i>	<i>M. nigra</i>	Hybrid
<i>M. tonkeana</i>	0.1571 (0.0548)	0.4725 (0.1101)	1.0830 (0.1838)	0.3318 (0.0703)
<i>M. maurus</i>	-0.0742 (0.0329)	0.1670 (0.0377)	0.8896 (0.1428)	0.1403 (0.0644)
<i>M. nigra</i>	-0.2071 (0.0526)	-0.1053 (0.0412)	0.5119 (0.0876)	1.1361 (0.1535)
Hybrid	0.0824 (0.0302)	-0.0084 (0.0242)	-0.2381 (0.0379)	0.1479 (0.0309)

¹ The diagonal represents the unbiased diagonal values of the estimated R matrix. Listing of unbiased minimum genetic distances (d^2) are found above the diagonal². The d^2 values listed were not pairwise-calculated. Standard errors are in parentheses. Hybrid represents *M. tonkeana*-*M. maurus* hybrid sample.

does not show any temporal or geographic patterning as was seen in the plot of Sulawesi macaques. The groupings indicated by the average linkage dendrogram are apparent along the first axis of the principal coordinate plot describing the first scaled eigenvector. Because each of the Paleolithic samples is comprised of individuals from differing geographic areas, the lack of any geographical or temporal patterning displayed by the principal coordinate ordination is not unexpected. Similar to the results of the average linkage cluster analysis, the Qafzeh sample (Qafzeh 6 and 9) is roughly positioned near the Upper Paleolithic sample in multivariate space.

Genetic divergence

The minimum genetic d^2 values calculated from the unbiased R matrix consisting of all four Paleolithic samples appears to be high (Table 5), ranging from 0.582 between the Lower Paleolithic and Middle Paleolithic samples, to 3.62 between the Lower Paleolithic and Upper Paleolithic samples. The minimum genetic d^2 distances calculated from the unbiased R matrix consisting of all the Sulawesi macaque taxa (Table 6), however, are much lower, ranging from 0.140 between *M. maurus* and its conspecific hybrid, to 1.136 between *M. nigra* and the *M. tonkeana*-*M. maurus* hybrid, indicating reduced genetic diversity compared to that observed among the Pleistocene hominids.

The observed minimum pairwise-calculated genetic distance between Middle Paleolithic Neanderthals and Upper Pleistocene modern humans ($d^2 = 2.496$) is significantly greater than that observed between the naturally hybridizing *M. maurus* and *M. tonkeana* ($d^2 = 0.6787$, $Z = 7.190$, $P < 0.0001$), as well as the distances observed between *M. nigra* and *M. maurus* ($d^2 = 1.388$, $Z = 4.114$, $P < 0.0001$), and between *M. nigra* and *M. tonkeana* ($d^2 = 1.354$, $Z =$

TABLE 7. Pairwise F_{ST} values describing within-grouping genetic differentiation for human and macaque samples included in this study, calculated using an average narrow-sense heritability of 0.55

Grouping	F_{ST}	Standard error
<i>M. maurus</i> - <i>M. tonkeana</i>	0.1541	0.0279
<i>M. maurus</i> - <i>M. nigra</i>	0.3184	0.0308
<i>M. tonkeana</i> - <i>M. nigra</i>	0.3321	0.0264
Combined macaque samples ($n = 4$)	0.2460	0.0207
MP-UP	0.5928	0.0183

MP, Middle Paleolithic; UP, Upper Paleolithic.

3.988, $P < 0.0001$), that cannot interbreed in the wild by virtue of their geographic separation.

Genetic differentiation

The F_{ST} values and their standard errors calculated from pairwise analyses using RMET are presented in Table 7. These values of F_{ST} represent unbiased measures of genetic differentiation calculated after correction for small sample size bias. Although not typically used as a measure of taxonomic divergence, Wright's F_{ST} should provide a reasonable measure of genetic differentiation within and among species. The combined Sulawesi macaque samples showed a surprisingly low unbiased F_{ST} value ($F_{ST} = 0.246$), given their morphological variability reported in the literature (see Albrecht, 1978). Their level of differentiation is, however, higher than what is observed among living human primates. For example, Relethford (1994) presents F_{ST} values ranging from 0.144 for six modern human populations, to 0.122 for three populations, using craniometric data. Under the assumption that the modern human population size was nine times greater than that of Neanderthals, Donnelly et al. (1998) presented an estimated F_{ST} value of 0.13 for

modern humans and Neanderthals using eight craniometric variables.

Based on our analysis assuming equal population sizes, the estimated F_{ST} value for the Neanderthal-Upper Paleolithic modern human grouping was comparatively high ($F_{ST} = 0.593$), indicating that these two congeners exhibit a greater genetic differentiation than that observed among the combined Sulawesi macaque samples comprised of three morphologically distinct species taxa and one hybrid sample. Our value is considerably higher than that presented by Donnelly et al. (1998), and is likely a product of differences between the two studies in assumptions regarding population sizes. Also, the Neanderthal-Upper Paleolithic modern human F_{ST} value is significantly greater than that observed for the macaque species grouping that are known to hybridize (*M. maurus* and *M. tonkeana*, $F_{ST} = 0.1541$; $Z = 13.148$, $P < 0.0001$), as well as greater than the geographically distinct species that do not hybridize (*M. nigra* and *M. tonkeana*, $F_{ST} = 0.332$; $Z = 8.116$, $P < 0.0001$, and *M. nigra* and *M. maurus*, $F_{ST} = 0.318$; $Z = 7.659$, $P < 0.0001$).

DISCUSSION

The morphological distinctiveness and presumed genetic divergence of the Middle Paleolithic Neanderthals reported by Turbón et al. (1997) are largely confirmed by the results of our analysis. The authors' assumptions regarding the genetic isolation of Neanderthals also appear to be valid, as does their assertion for a monophyletic origin of modern humans in Europe separate from Neanderthals. Our comparison of estimated F_{ST} values, calculated with a narrow-sense heritability of 0.55, shows that the genetic differentiation for the Upper Paleolithic modern human-Middle Paleolithic Neanderthal grouping is significantly greater than that observed for the Sulawesi macaque taxa that are known to hybridize fully in the wild (i.e., *M. tonkeana* and *M. maurus*), as well as greater than those observed between the noninterbreeding Sulawesi taxa (i.e., *M. tonkeana* or *M. maurus* with *M. nigra*).

If *M. tonkeana* is designated as a subspecies of *M. maurus*, as would seem necessary under the biological species concept, then the genetic divergence and differentiation observed for the Neanderthals and the Upper Paleolithic modern humans might, or might not, be taxonomically significant at the species level. However, when we compare the genetic divergence and differentiation observed between Neanderthals and Upper Paleolithic modern humans with those seen between Sulawesi macaque species that cannot interbreed, a species-level distinction seems warranted.

Based on the results of the significance tests comparing d^2 and F_{ST} values between the Pleistocene hominid and Sulawesi macaque samples, we were unable to reject the hypothesis that the observed genetic divergence and differentiation between Neanderthals and Upper Paleolithic modern humans

are greater than those between naturally hybridizing Sulawesi macaque taxa. These results, therefore, seem to support the replacement model for the origin of modern humans in Europe by indicating that the level of genetic divergence and differentiation seen between Upper Paleolithic modern humans and Middle Paleolithic Neanderthals is greater than that for the morphologically diverse extant nonhuman primate taxa that interbreed in the wild. These results, however, do not agree with previous research comparing morphological variation observed among extant naturally hybridizing nonhuman primate species with Pleistocene hominids (i.e., Simmons, 1994, 1999; Simmons and Smith, 1991), nor are they consistent with the recent discovery of a possible Neanderthal-modern human hybrid on the Iberian Peninsula (see Duarte et al., 1999).

Limitations to the study

We temper these conclusions with the realization that the Paleolithic hominid samples included in our analysis are small and originate from geographically and temporally dispersed sites across Europe, the Middle East, and Africa, and therefore were not randomly mating (or even potentially interbreeding) populations (see Norborg, 1998). The Sulawesi macaque samples, moreover, originate from a much smaller geographic area, and likely represent no more than five or six generations. In addition, because the Upper Paleolithic modern human-Middle Paleolithic Neanderthal grouping consists of specimens from different time periods, the observed large F_{ST} values might merely represent temporal changes in genetically determined within-taxon craniofacial morphology. Therefore, the significant differences in the observed divergence and genetic differentiation between the macaque and Paleolithic samples might be, at least in part, a function of differences in their geographical and temporal makeup.

Because it has been shown that demographic factors, such as increases or decreases in effective population sizes, can influence genetic differentiation (see Relethford, 1991; Relethford et al., 1997), it is important to consider the potential effects of Pleistocene Middle and Upper Paleolithic population sizes on F_{ST} values. Based on recent estimates, it seems likely that Upper Paleolithic population sizes were considerably larger than Middle Paleolithic Neanderthal populations during the Late Pleistocene (reviewed in Relethford, 1998). Furthermore, decreasing population sizes among Neanderthals would increase their within-group, or -taxon, differentiation due to genetic drift, potentially increasing the F_{ST} value for our Upper Paleolithic modern human-Middle Paleolithic Neanderthal grouping. Such an increase would not necessarily be a product of any phylogenetic or taxonomic differences between these two groups, but instead would reflect differences in demographic structure.

Fig. 6. Line graph describing relationship between Middle Paleolithic Neanderthal relative population sizes and genetic differentiation, as measured by unbiased estimates of F_{ST} . Error bars represent $\pm$ standard error.

In order to estimate the effects of unequal effective population sizes on genetic differentiation within our Upper Paleolithic modern human-Middle Paleolithic Neanderthal grouping, we generated a series of F_{ST} values using different relative weightings in RMET. The results presented in Figure 6 indicate that differences in relative population sizes do affect the magnitude of genetic differentiation, with smaller relative Neanderthal population sizes being associated with lower F_{ST} values for this grouping. This relationship between relative population size and genetic differentiation was highly significant (Pearson $r = .938$; $P = 0.006$). Despite this relationship, however, the lowest relative Neanderthal population size (5%:95%) was associated with a considerably higher F_{ST} value ($F_{ST} = 0.4874$) than that observed for the hybridizing and nonhybridizing Sulawesi macaque taxa calculated using equal weighting (i.e., 50%:50%) (see Table 7). This finding supports our conclusions that mate recognition between Neanderthals and Upper Paleolithic modern humans might not have been possible.

Despite these limitations to our study at the population level, minimum genetic distances and Wright's F_{ST} are likely reasonable measures of genetic divergence and differentiation between and among species-level taxa. The taxonomic designation of the Paleolithic samples included in this analysis, whether valid at the species or subspecies level, is based on shared patterns in overall craniofacial morphology that distinguish them from other groups (see Turbón et al., 1997, for their rationale regarding taxonomic designations). While it is true that these samples are not reproductive populations, we be-

lieve they represent reasonable hypothetical taxonomic groupings suitable for morphometric and minimum genetic comparisons.

It should also be considered that although the Sulawesi macaques are variable within their genus, *Macaca* as a whole may be craniometrically monomorphic when compared to the genus *Homo*. In addition, the geographical space separating *M. nigra* from the hybridizing *M. tonkeana* and *M. maurus* is occupied by two other hybridizing taxa, allowing for the possibility of gene flow between our samples (see Watanabe and Matsumura, 1991). Other nonhuman primate genera such as *Papio* might be more appropriate for studies comparing within-genus craniofacial variation. For example, despite more than 3.5 million years of evolutionary divergence, the morphologically distinct *Theropithecus gelada* and *Papio hamadryas* have been reported to occasionally interbreed in the wild, producing fertile hybrid offspring (Jolly et al., 1997). This particular example of nonhuman primate hybridization might best approximate the ecological, behavioral, genetic, and morphological diversity seen between Neanderthals and Upper Paleolithic modern humans, with minimal hybridization allowing the spread of significant genes (cf. Jolly et al., 1997).

The present study is merely the first attempt at using minimum genetic distance and divergence of extant naturally hybridizing nonhuman primates as a tool for reexamining the hominid fossil record. Future research incorporating morphologically, behaviorally, and ecologically more disparate hybridizing primate taxa such as *Papio hamadryas hamadryas* and *P. h. anubis* (see Simmons, 1999), or even *Theropithecus gelada* and *Papio hamadryas*, as well as geographically and temporally more focused Pleistocene samples, is needed.

CONCLUSIONS

The determination of taxonomic and evolutionary significance for the morphogenetic divergence observed in the human and nonhuman primate fossil record should be based on empirical studies of present-day biological processes such as primate hybridization. Only by examining the level of morphological divergence associated with maintaining pre- and postmating isolation between extant sympatric or parapatric taxa, and by understanding that isolating mechanisms involving morphology, such as SMRS, can break down and result in limited or even full introgression, can we begin to interpret the evolutionary history of fossil primates, hominid or otherwise.

The present study shows that the genetic divergence and differentiation between Middle Paleolithic Neanderthals and Upper Paleolithic modern humans, as indicated by minimum genetic distances d^2 and F_{ST} values calculated from the estimated minimum R matrix, are likely taxonomically significant at the species level when compared with the genetic divergence and differentiation of hybridizing

and nonhybridizing macaque taxa from Sulawesi, Indonesia. These results, which are congruent with the recent molecular evidence indicating that Neanderthals are genetically distinct (Krings et al., 1997, 1999; Ovchinnikov et al., 2000; Scholz et al., 2000), indicate that a conspecific designation for Neanderthals and modern humans may not be warranted (but see Norborg, 1998; Relethford 1998). Furthermore, the lack of measurable gene flow between Neanderthals and Upper Paleolithic modern humans supports assumptions regarding the monophyletic origins of modern humans outside Europe and independent of Neanderthals.

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