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INTERACTIONS OF NEANDERTHALS AND MODERN HUMANS: WHAT CAN BE INFERRED FROM MITOCHONDRIAL DNA?

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Abstract. This paper reviews the state-of-the-art knowledge concerning the relationship between Neanderthals and Upper Paleolithic modern humans. The branching-process method is applied to infer the upper limit of hypothetical Neanderthal admixture, consistent with the evidence based on mitochondrial DNA sequences of contemporary modern humans, as well as Neanderthal and early modern European *H. sapiens* fossils. As a result, a maximum value of 15% admixture is obtained. This estimate is discussed in the context of its consequences for the two competing theories of modern human origin.

1. **Introduction.** In the past decade, several important discoveries were made concerning the origin of our species. These discoveries include fossils dated several million years ago, such as a skeleton of *Pierolapithecus catalaunicus* (an early great ape from the middle Miocene [1]) and, a few million years younger, skeletons of *Sahelanthropus tchadensis*, *Orronin tugenensis*, *Ardipithecus ramidus*, and *Australopithecus anamensis*. The latter four are claimed to be our extinct ancestors living in the Pliocene [2, 3]. The discoveries also include the LB1 skeleton of *Homo floresiensis* [4], which flourished as recently as 18,000 years ago. The latter is especially intriguing, as it is a representative of the genus *Homo* probably different from our own species and being alive in Indonesia during the late Pleistocene, only about 38,000–18,000 years ago [5]. Because of its height (approximately 1 m), and the size of its brain (about 380 cm^3), *H. floresiensis* exhibits the most extreme case of the genus *Homo* and hardly matches any of previously considered interpretations of human origins.

It will require some time and perhaps new discoveries to give a coherent explanation of *H. floresiensis* within the framework of rewritten hypotheses of human origin. The early conclusions of evident isolation of small-bodied humans seem to contradict the multiregional hypothesis. Mirazon Lahr and Foley [6] express this fact more strongly when writing in *Nature* that "*H. floresiensis* puts yet another

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(the last?) nail in the multiregional coffin."Yet multiregionalists remain skeptical. They claim continuity of anatomical features provides strong paleoanthropological support for multiregional evolution of humans, especially in Asia but also in Australia and Europe, before and after the arrival of modern humans from Africa [7]. Indeed, assuming no interbreeding between archaic (autochthon) and modern (invader) humans, it is difficult to explain the fact that some bone features of Australians, clearly distinctive from African, are present in Australian fossils before and after the appearance of modern humans in the region. Tattersall [8] disagrees with this interpretation and considers *Homo erectus* a local evolutionary dead end, whereas Cann and Wilson [9] indicate that the bone features mentioned are not necessarily independent and selectively neutral. They suggest that successive reevolution of similar bone patterns is plausible in similar environmental conditions. Still, the relatively short time required for the replication of changes makes this explanation at least disputable, especially bearing in mind that some nuclear genes support migration and evolution patterns different from those inferred from mitochondrial DNA (mtDNA) [10].

Nevertheless, because of the ease with which the polymerase chain reaction (PCR) amplifies mtDNA present in multiple copies in each cell and the fact that mtDNA does not recombine, mtDNA-based inferences are an important source of knowledge about the origin of modern humans. This is even truer in light of conflicting inferences based on different autosomal microsatellite loci. Estimates of Kimmel et al. [11] may be interpreted as suggesting that extensive population growth had occurred in Asia and Europe but not in Africa, whereas Reich and Goldstein [12] inferred just the opposite.

Successful sequencing of mtDNA from Neanderthal fossils became the milestone in revealing our evolutionary paths. For example, until recently, estimation of the mitochondrial mutation rate relied only on human-chimpanzee divergence data. Because of the relatively long time over which this divergence occurred, all estimates of this time were very inaccurate, ranging from 4 million [13] to 9 million years [14]. As a consequence, the estimated mutation rate could not be accurate; this affects the estimates of the age of mitochondrial Eve (mtEve), the most recent female carrier of mtDNA ancestral to that of all modern humans. O'Connell [15], based on genetic diversity of modern humans applied to his branching-process model, estimated the age of mtEve as ranging between 700,000 to 1.5 million years.

O'Connell's results were very different from those obtained with the use of phylogenetic trees. Hasegawa and Horai [16] and Wilson and Cann [17] estimated the age of mtEve as 280,000 and 200,000 years, respectively. Until recently, summarizing mtEve dating estimates depended on inaccurate inferences about human-chimpanzee divergence time, and furthermore, they mostly depended on the method of inference applied.

The situation changed after 1997 [18], when for the first time the mtDNA from a *Homo neanderthalensis* dated to be alive about 40,000 years ago [19] was sequenced. However, less than 400 base pairs were sequenced; hence, any estimates based on these data were not very reliable. The next successful sequencings of Neanderthal mtDNA [20, 21, 22] confirmed the accuracy of the first experiment and qualitatively changed the situation in dating the root of mtDNA polymorphism of contemporary humans. The mtDNA divergence rate no longer has to be guessed by relying on the assumption of its constancy over a few million years and on the problematic dating of the human-chimpanzee split.

In 2004, four additional Neanderthal fossils and five early modern human fossils yielded mtDNA sequences [23], and the results were fully accorded with previous sequencing efforts. Also important, fossils sequenced by Serre et al. (2004) contained examples (specimens labeled Vandija 77, Vandija 80, Mladeč 25c, and Mladeč 2) considered, by multiregionalists, as transitional between Neanderthals and early modern humans based on some morphological features [24, 25, 26, 27]. Therefore, it could be possible that Vandija fossils considered as Neanderthal would yield modern human mtDNA, or Mladeč fossils, considered as modern human would yield Neanderthal mtDNA. Yet the mtDNA proved to be of Neanderthal type for Vandija fossils and of modern human type for Mladeč fossils, and no transition evidence has been detected. This is exactly what is expected by the out-of-Africa model, suggesting that some morphological features shared by the fossils mentioned could be results of similar environmental influences or could arise by chance, without a strong genetic flow between Neanderthals and early modern humans.

Serre et al. [23], apart from reporting these results, tried to estimate the upper limit of possible Neanderthal admixture in early modern humans, consistent with mtDNA evidence. They used a coalescence method in three different demographies: (i) constant population size and population growth, (ii) before the potential point of Neanderthal admixture, and (iii) after the potential point of Neanderthal admixture. The numerical value of the estimate equal to 25% is given only for the simplest case of population constant size, which however is known to be unrealistic.

In this paper, we estimate the upper bound on Neanderthal admixture using the branching-process model. This approach was pioneered by O'Connell [28, 15]. Tavaré et al. [29] recently used a similar approach to infer the age of the last common primate ancestor, based on archeological stratification and the number of species known to have lived in a given period.

Results we obtained reduce the maximum hypothetical Neanderthal mtDNA admixture to the early modern human gene pool to about 15%. More accurate estimates will be possible when the archeological studies correlating Aurignacian, Chatelperronian, and Gravettian cultures with Neanderthals or modern humans [30], as well as the influence of the Ice Ages on demography [31], yield more reliable estimates of the population size in different regions of the globe and corresponding time-inhomogeneous branching processes are used.

2. **Models, data and simulations.**

2.1. **Models.**

The first stochastic model we consider was proposed by O'Connell [15] for dating mitochondrial Eve's death, based on a sample of mtDNA of humans and chimpanzees. The validity of his model relies on the assumption that the population is growing as a slightly supercritical branching process, with progeny distributions homogeneous in time. Asymptotically, for a given expected number of offspring, O'Connell's model is independent of the precise form of the offspring distribution as long as the variance of the distribution is bounded. This is not so for Wright-Fisher (W-F) models [32], the second model considered in this paper, which always assume multinomial sampling from one generation to the next. On the other hand, W-F models, contrary to O'Connell's model, are not limited to any specific growth patterns. Since W-F models are well known, we will focus on the description of O'Connell's model only.

2.1.1. *O'Connell's model.* Consider a family of slightly supercritical time-homogeneous Markov branching processes with expected numbers of offspring per individual equal to $E(\xi_0) = 1 + \alpha/T + o(1/T)$ and the corresponding variance equal to $Var(\xi_0) = \sigma^2 + O(1/T)$, as $T \to \infty$. This family is indexed by T, and any particular branching process belonging to this family is defined for a large but fixed time T. Let us denote the number of individuals in the process at time t by Z_t. The probability of nonextinction $P^x(Z_t > 0)$, where P^x denotes probabilities starting the process from x individuals, is given by O'Connell [15]:

$$P^x\left(Z_t > 0\right) \sim \frac{2\alpha x}{\sigma^2 T}\left[1 - \exp\left(-\alpha\frac{t}{T}\right)\right]^{-1}, \qquad as\ T \to \infty. \tag{1}$$

This equation describes the rate of approaching zero by the probability of nonextinction of the process until time t, for T tending to infinity. Parameter T, indicating the particular branching process in a family, can be interpreted as the duration of the respective process expressed in time units of the average generation. In our approach, the process Z_t represents the number of Neanderthal mtDNA genomes present in the admixed population of modern humans, at time t from the moment of admixture.

Our purpose is to use the relationship above to develop an estimate of x, interpreted as the number of Neanderthal mtDNAs present in the admixed population of early modern humans, such that the probability of nonextinction until present would be 95%. It can be observed from equation (1) that the validity of this asymptotic property holds for finite but large times T, as long as the probability of nonextinction of descendants of more than one initial individual is negligible: then the probability of nonextinction of x independent branching processes starting from one individual is nearly equal to the sum of x identical probabilities of nonextinction of one branching process starting from one individual. That is only true for small values of $P^x\left(Z_T > 0\right)$ (we use $t = T$, since we are interested in nonextinction until T, i.e., until present). However, our goal is to calculate the number of Neanderthal individuals x yielding the probability of nonextinction of their mtDNA lineage $P^x\left(Z_T > 0\right) = 0.95$. Then, having the information that all Neanderthal sequences have disappeared until now, we can conclude that (with probability 95%) the actual number of Neanderthal sequences in a human gene pool about 30,000 years ago had to be less than x. The conclusion can be drawn from the observation that the corresponding branching process reflecting the hypothetical process of preservation of Neanderthal mtDNA sequences, starting from a given number of Neanderthal sequences x, has become extinct for no more than 5% of cases.

To obtain x, first we used equation (1) to calculate the probability of nonextinction $P^1\left(Z_T > 0\right)$ of the branching process starting from one individual. Then we computed the value of x for which $P^x\left(Z_T > 0\right) = 0.95$, observing that the probability of extinction of the process started from x individuals is equal to the joint probability of extinction of all x subprocesses, each started from one individual. The union of the resulting subprocesses constitutes the original process. Hence, $1 - P^x\left(Z_T > 0\right) = \left[1 - P^1\left(Z_T > 0\right)\right]^x$ and condition $P^x\left(Z_T > 0\right) = 0.95$ is equivalent to $\left[1 - P^1\left(Z_T > 0\right)\right]^x = 1 - 0.95$. From this, it follows that

$$x = \frac{\ln\left(1 - 0.95\right)}{\ln\left(1 - P^1\left(Z_T > 0\right)\right)}. \tag{2}$$

The parameter of the expected growth rate, equal to $1 + a/T = 1.001$, was chosen based on general human population growth starting from mtDNA Eve time, yielding the final inbreeding effective population size two orders smaller than the actual size. We assumed the feasible variance σ^2 of the offspring distribution to be as large as 10 (compared to 1 for Poisson distribution). This conservative choice seems somewhat arbitrary, but without knowing the actual demography, we preferred to be conservative. A higher (i.e., more likely to be true) value of α (or a smaller value of σ^2) would result in an actual value of x smaller than what we have estimated here, and consequently, the maximum Neanderthal admixture consistent with mtDNA evidence should have been further reduced. Implicitly, we assume here that the average fitness of the postadmixture individuals carrying Neanderthal mtDNA is equal to that of African mtDNA carriers. However, if this assumption is not true – that is, if the admixed individuals are less fit than mtEve descendents – then it is automatically the explanation of the replacement, claimed by multiregionalists to be false [7].

To estimate the age of mtEve (expressed in generations T, or in years $T_a = \lambda T$, assuming that the duration of the average generation is equal to λ years), we used a combination of the O'Connell model and the W-F model of genetic drift conditioned on the population size evolving as a slightly supercritical branching process. The W-F model, probably the most widely used model in population genetics studies, has been well explored for deterministic demographies. Here we apply it to stochastic demography modeled by a branching process and compare the results with O'Connell's predictions obtained from asymptotic properties of branching processes. We should point out that other approaches exist, based on coalescence theory and phylogenetic trees, but they are not considered here.

Let us express the time interval $[0, T]$ of a variable t as a unit interval $[0, 1]$ of variable $r = t/T$. Furthermore, let us again consider a family of slightly supercritical time-homogeneous Markov branching processes with the expected number of offspring $E(\xi_0) = 1 + \alpha/T + o(1/T)$ and variance $Var(\xi_0) = \sigma^2 + O(1/T)$. We assume one member of this family approximates the long-term history of human population. Then, for long times T, we have the following equation describing the tail of the distribution of D_T, the time of death of the last common ancestor of a pair of individuals alive at T (i.e., the present), given that we start the population history from x individuals having descendants at T (see also [15] and the corretion apearing in: [33]):

$$P\left(D_T > rT \,|N_T = x\right) = \frac{2q_r^x}{(x-1)!}\left[-\left(1-q_r\right)^{-x}(x-1)! - F\left(x-1, 1-q_r\right)\right], \quad (3)$$

where

$$q_r = \frac{e^{-r\alpha} - e^{-\alpha}}{1 - e^{-\alpha}} \quad (4)$$

and $F : \mathbb{Z}_+ \times (0,1) \to \mathbb{R}$ is defined as

$$F\left(n, y\right) = \frac{\partial^n}{\partial y^n}\left[\frac{\ln\left(1-y\right)}{y^2}\right]. \quad (5)$$

O'Connell's original distribution is continuous, but to compare it with discrete empirical distributions obtained from simulations, we consider the discretized version, specified by the tail of original distribution computed at points r corresponding to integer values of $t = rT$.

Let us denote the average genetic distance in a sample of n DNA sequences by d_{avg} and the genetic divergence rate by δ. Furthermore, let T_c be the time of coalescence (i.e., the time counted from present to the last common ancestor) of the pair of alleles. Then, for $n << Z_T$,

$$E\left(d_{avg}\,|N_0 = x\right) = \delta\lambda E\left(T - D_T\,|N_0 = x\right) = T\lambda E\left(\frac{T_c}{T}\,|N_0 = x\right), \tag{6}$$

and therefore the moment-based estimate for T_a is

$$\widehat{T}_a = d_{avg}\left[dE\left(\frac{T_c}{T}\,|N_0 = 1\right)\right]^{-1}. \tag{7}$$

In O'Connell's model, to calculate $E(T_c/T\,|N_0 = 1)$, we need α; however, we can simultaneously estimate T (or equivalently T_a) and α. In a process started from mitochondrial Eve (one individual), the expected size of the (nonextinct) population at present is given by

$$E\left(Z_T\,|Z_T > 0\right) = \frac{E\left(Z_T\right)}{P^1\left(Z_T > 0\right)}, \tag{8}$$

where Z_T reads as the present population size of humans. Since

$$E\left(Z_T\right) = \left(1 + \frac{\alpha}{T}\right)^T \approx \exp\left(\alpha\right), \tag{9}$$

it follows from (1), (8), and (9) that

$$Z_T \approx \frac{\sigma^2\widehat{T}_a}{2\lambda\widehat{\alpha}}\left[\exp\left(\widehat{\alpha}\right) - 1\right]. \tag{10}$$

Estimates of T_a and α are solutions of the system of equations (7) and (10). In W-F stochastic models, $E(T_c/T\,|N_0 = 1)$ must be computed numerically from simulations.

2.2. **Genetic data.** We used the average pairwise genetic distance data between contemporary *Homo sapiens* and *Homo neanderthalensis* as published by Krings et al. [20]. For the sample of 689 modern humans, the average pairwise number of segregating sites in DNA taken from the hypervariable control regions I and II of the total length 600 nucleotides was equal to 35.3 ± 2.3. Therefore, the average genetic distance was equal to $d_{agvM-N} = 5.9\%$. The distribution of pairwise divergence in contemporary humans resulted in an average number of segregating sites 10.9 ± 5.1, and thus the average genetic distance among contemporary humans was equal to $d_{avg} = 1.8\%$. It is less than the value of 2.8% reported by O'Connell [15], however, for a much smaller sample of 19 humans. The average genetic difference between Neanderthals and modern humans, about three times greater than that calculated within contemporary humans, is still small enough to ignore reverse mutations occurring in both lineages from the time of their divergence T_d some 500,000 years ago [20]. Therefore, by applying the infinite site model (for details, see [32]), we can calculate the rate of divergence as $d = d_{avgM-N}/T_d \approx 0.06/500,000 = 1.2 \times 10^{-7}$.

This estimate is within the confidence interval $[5.9 \times 10^{-8}, 1.4 \times 10^{-7}]$ reported by Adachi and Hasegawa [34].

2.3. **Simulations.** To compare distributions of the time to coalescence of a pair of alleles, we performed numerically more than 10^5 simulations of human population trajectories covering a period of 10^4 generations (the simulation took more than three weeks using a Pentium IV with HT Technology, 3.06 GHz). For a human generation length of approximately 20 years, 10^4 generations is equivalent to 200,000 years, covering the approximate interval from the mitochondrial Eve until the present. These simulations are needed to obtain parameter α from a W-F model, conditioned on the branching-process population scenario. To perform so many simulations in an unbiased way, the built-in pseudo-random number generators were not sufficient because of a too-short range of aperiodicity or a failure to satisfy the overlapping pairs sparse occupancy (OPSO) test [35]. To overcome the problem, we implemented a generator [36, 37] composed of a Fibbonacci generator with a period of 2^{120} and a generator with a period of $2^{24}-1$. The resulting generator, with a period length of 2^{144}, satisfies all known statistical tests and is invariant with respect to the internal number representation (assuming at least 16-bit integers and 24-bit mantissa for floating-point arithmetics, which is always fulfilled in contemporary computers).

The estimated maximum number of Neanderthal mtDNA sequences in the gene pool of early modern humans, consistent with the absence of such sequences in a gene pool of contemporary humans, was computed using probabilities of extinction of a branching process starting from one individual as obtained from O'Connell's theory [28, 15]. The required parameter α of a stochastic growth rate was obtained from simulations aimed at dating mtEve as reported in [38]. Here we assumed this parameter to be conservative for the purpose of our estimations and set $\alpha = 10$ in equation $E(\xi_0) = 1 + \alpha/T + o(1/T)$. This means that we assumed a branching process with the growth rate smaller than that leading to the actual short-term inbreeding effective population size by two orders of magnitude. In this way, we tried to assure that possible periods of adverse environmental effects have no significant influence on the results obtained.

3. **Results.** In Table 1, we summarize estimations of the relative time of coalescence of a pair of alleles with respect to total population history length T. This table presents moments of this distribution (columns 2–4), a comparison of the Kolmogorov-Smirnov test for equality of distributions with distribution in O'Connell's model (last column), and final (absolute, not effective) population sizes for the assumed value of $\alpha = 10$. Table 2 gives relative departures of estimates of the mtEve age, with respect to the estimate based on O'Connell's model. The time to the most recent female ancestor of contemporary humans expressed in years equals $T_a = \lambda T$. The estimates of this time, assuming $\delta = 1.2 \times 10^{-7}$ and $d_{avg} = 0.018$ for different population histories, are given in Table 3.

Comparison of Table 3 with the 95% confidence interval $[111 \times 10^3, 260 \times 10^3]$ [20] shows that all stochastic model predictions fall into it, although particular coalescence time distributions (see Table 1) significantly deviate from O'Connell's distribution according to the Kolmogorov-Smirnov test. That means that the predictions of the W-F models are not very sensitive to actual departures from the assumption of multinomial sampling, though statistically these departures significantly change the distributions of the time to coalescence.

TABLE 1. Estimation of relative time to coalescence of a pair of alleles for different population histories

Population trajectory scenario	Expectation of $\frac{T_c}{T} \mid N_0 = 1$	Standard deviation of $\frac{T_c}{T} \mid N_0 = 1$	Final population size	Equal to O'Connell's distribution?
O'Connell's	0.801	0.159	10^7	—
W-F: P	0.802	0.159	10^7	Yes
W-F: BF	0.735	0.170	0.5×10^7	No
W-F: LF	0.844	0.156	2.0×10^7	No
W-F: P, σ_{e1}	0.794	0.170	10^7	Not sure
W-F: P, σ_{e2}	0.699	0.269	2.0×10^7	No

Note: The genealogy starts with one individual and ends with the number indicated in the fifth column. The first column defines the population trajectory scenario: P, Poisson offspring distribution; BF, binary fission offspring distribution; LF, linear fractional offspring distribution. Symbols σ_{e1} and $\sigma_{e2} = 3 \times \sigma_{e1}$ denote standard deviations of expected number of progeny, varying from generation to generation in a branching process nonhomogeneous in time (also called the branching process in a varying environment).

TABLE 2. Ratios of standardized estimates of the date of death of the last common ancestor for a given distribution and O'Connell's distribution

$$\frac{E(T\lambda\delta/d_{avg})}{E_{O'Connell}(T\lambda\delta/d_{avg})}$$

O'Connell's	W-F				
	time-homogeneous			time-inhomogeneous	
	P	BF	LF	P with σ_{e1}	P with σ_{e2}
1	0.999	1.091	0.950	1.009	1.147

TABLE 3. Estimates of the time to mitochondrial Eve

$$E\left(T_c\right) \quad [10^3 \text{ years}]$$

O'Connell's	W-F				
	time-homogeneous			time-inhomogeneous	
	P	BF	LF	P with σ_{e1}	P with σ_{e2}
187	187	204	178	189	215

For the W-F model with population size modeled by a branching process reflecting the average growth rate of human population characterized by values $\alpha = 10$ and $T = 10,000$, we calculated $P^1\left(Z_T > 0\right) = 0.002$. Assuming further that the

fitness of individuals admixed by Neanderthal mtDNA is on average equal to that of African mtDNA descendents, we determined from (2) that $P^x(Z_T > 0) = 0.95$ for $x \approx 1,500$. This value represents a short-term inbreeding effective subpopulation size of Neanderthal mtDNA carriers in the postadmixture human population. The corresponding short-term inbreeding effective population size of the whole human mtDNA population at this time (about 30,000 years ago) can be estimated to be at least 10,000. This result is obtained if we assume the minimum of estimates of census population sizes equal to 500,000 [39], which gives a 100,000 census population size of females active in reproduction, provided half of the population is composed of females and that living females represent 2.5 generations. To obtain the short-term inbreeding effective population size, this number must be further divided by the variance of the distribution of the number of daughters of an individual female, a figure that is presumably less than 10. Conservative values presented result in an amount of possible Neanderthal mtDNA admixture of less than 15%.

4. **Discussion.** As long as some known facts are difficult to understand in the light of a hypothesis, an alternative to this hypothesis cannot be disregarded. This is why, instead of trying to disprove either the multiregional or the out-of-Africa model, both of which have difficulty in explaining some known facts, we draw conclusions, that may make any of these models more reliable. However, the consequences of our inference are not equivalent for these two (active) competing hypotheses. For the out-of-Africa scenario, our results quantitatively show to what extent this scenario can rely on mtDNA inferences. It is important in drawing conclusions, should a morphological fossil record contradicting the pure version of the out-of-Africa hypothesis be discovered. In other words, even if to-date mtDNA-based results do not contradict the radical form of the recent out-of-Africa origin theory, they cannot be treated as a proof of the model of total replacement. Total replacement of archaic *Homo* populations by descendents of mitochondrial Eve, without any admixture from archaic autochthon genes, is unlikely in the light of fossils, as multiregionalists often emphasize.

On the other hand, indicating the insufficiency of mtDNA-based inferences is not equivalent to ignoring them and treating mtDNA and molecular clock methods as entirely unreliable. Since mtDNA, recently sequenced from Neanderthal and ancient *Homo sapiens* fossils, can be the basis for estimating the upper limit of plausible Neanderthal mtDNA contribution in descendants of Eve, the predictions of the multiregional model cannot dramatically exceed these limits. Limits of admixture have been bounded by about 25% [23], and we further reduce the extent of plausible Neanderthal mtDNA contribution applying another strategy. Our method, based on a branching-process model, allowed us to calculate the limit of Neanderthal mtDNA admixture, which with a probability of 95% would have been preserved until present if it had been added to the human gene pool about 30,000 years ago. That was the time when the Neanderthals probably disappeared, but the question arises whether and how much they contributed to modern human genes before extinction.

Thorne and Wolpoff [7] suggest that this contribution should be up to 50% in an early population of modern humans in Europe and that genetic drift cleared any trace of this fact in contemporary humans. These authors also claim that mitochondrial inference concerning Neanderthals is unreliable because of the contamination of mtDNA from Neanderthal fossils by contemporary sequences. At the opposite

side, some supporters of the out-of-Africa model treat the mtDNA evidence as the evidence of no Neanderthal mtDNA contribution at all. Below, we briefly discuss these issues in view of our results.

Our results show that Thorne and Wolpoff [7] present an extreme but not necessarily false opinion concerning the amount of Neanderthal admixture. Our conservative assumptions led us to a maximum level of admixture of about 15%, with 95% confidence. However, this estimate was calculated as the proportion of Neanderthal mtDNA in the whole human population; Thorne and Wolpoff [7] mean the proportion in Europeans. The change from 15% in total population to 50% in Europeans, as postulated above by these multiregionalists, requires that Europeans constitute at most 30% of the whole population. This seems feasible, and therefore admixtures of similar magnitude can be accepted in light of the current knowledge. However, we must stress that in our calculations we have used the minimum estimate of the human population size 30,000 years ago. The maximum estimate is more than 10 times larger [39], and if this second estimate proves more likely, then the maximum Neanderthal admixture estimated in this paper will decrease 10 times, disproving claims about 50% Neanderthal admixture in early Europeans.

Possible contamination by modern sequences of ancient Neanderthal DNA probably would not yield sequences noticeably different from contemporary modern humans; yet this is exactly what occured. If they were really contaminated, then it means that in reality the genetic distance between *H. sapiens* and *H. neanderthalensis* would have been even greater than the estimation of genetic distance based on Neanderthal sequencing studies. Therefore, possible contamination (if present) supports the out-of-Africa model, predicting greater genetic distances between modern humans and Neanderthals as compared to multiregionalism predictions.

The percentage of Neanderthal mtDNA admixture, which cannot be excluded based on sequencing studies, estimated by Serre et al. [23] to be about 25%, should not be treated as the evidence of no contribution at all. Yet some important papers (mainly the older ones, such as Krings et al. [18, 20] and Ovchinnikov et al. [21]), when announcing the fact that the contemporary mtDNA gene pool does not contain mitochondrial genes inherited from Neanderthals, seem to neglect the effect of genetic drift, which, together with overstating the conclusions drawn from the lack of regional affinity of Neanderthals with contemporary Europeans, was criticized by Relethford [40]. Our results, although further reducing the plausible maximum amount of admixture to 15%, still cannot be used as a proof of no admixture. They do not, however, contradict such a radical form of replacement.

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