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Response: Rampino (in his letter) and Knoll *et al.* (in their response) attempt to refute our proposition (*1*) that Late Permian marine invertebrate mass extinctions coincide with the widespread development of marine anoxia and dysoxia. Their arguments are based on diametrically opposed interpretations of the Permian-Triassic deep sea record of Japan, and both rely heavily on the data from the same paper (*2*). Thus, neither letter challenges the well-established fact that most Late Permian marine invertebrates disappeared in shallow marine settings at levels marked by the appearance of dysaerobic or anaerobic biofacies (*3*). Rampino notes that the decline of radiolarians in Japanese sections occurs within a thin, siliceous claystone of latest Permian age immediately beneath a thin, basal Triassic organic-rich mudstone—a dysaerobic facies (*2*). He therefore argues that the extinction (of radiolaria at least) occurred before the development of oxygen-poor conditions. However, he does not mention the evidence of dysoxic conditions in the claystone, namely, common micronodules of pyrite and discontinuous lamination (*2*).

Conversely, Knoll *et al.* acknowledge the oxygen-poor conditions recorded by the claystone (and the several meters of bedded, grey chert developed beneath this level) and argue that the organic-rich mudstone records improved oxygenation. This is counter to Kakuwa's (and our) interpretation that this layer was a dysaerobic facies (*2*) and is primarily based on the presence of burrows in the mudstone and the interpretation of pyrite sulphur isotope variations (*4*). Kakuwa only illustrated millimeter-sized burrows from the claystone and mudstone, but did not document trace fossils from the underlying grey cherts.

Our observations of the chert ichnofabrics reveal them to be pervasively bioturbated by centimeter-sized burrows, testimony to substantially better benthic oxygen values than those of the organic-rich mudstone. The ichnofabrics, therefore, reveal a story of gradually declining benthic concentrations of O in the Late Permian record of Japan, culminating in low dysoxic conditions at the Permian-Triassic boundary. Identical changes are seen in contemporaneous shelf sections (*5*). Pyrite $\delta^{34}S$ variations show a sharp negative swing in the organic-rich mudstone (*4*), which Knoll *et al.* interpret as a signature of a fully oxygenated water column. However, strongly negative values of pyrite sulphur (S) can also indicate intensely anoxic conditions such as those pertaining in the present-day Black Sea, where sulphide disproportionating bacteria repeatedly process and lighten elemental S (*6*). Sulfur isotopes of evaporites provide more conclusive, less equivocal evidence of global changes in the S cycle. These reveal a rapid positive swing beginning in the latest Permian and continuing into the Early Triassic (*7*), which indicates a major phase of pyrite burial and oceanic anoxia. This evidence alone seems sufficient to rule out the scenario of oceanic overturn and ventilation proposed by Knoll *et al.* (*8*).

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HLA Sequence Polymorphism and the Origin of Humans

In the paper, "The myth of Eve: Molecular biology and human origins" (*1*), Francisco J. Ayala has made some questionable inferences about the origin of the human species based on analyses of mitochondrial DNA (mtDNA) and human lymphocyte antigen (HLA) sequence polymorphism. Ayala (i) argues that the data on primate HLA class II sequence diversity contradicts the "Mitochondrial Eve" hypothesis (*2*) about modern human origins, and (ii) estimates that the size of the founding human population was at least 100,000, primarily on the basis of assumptions about the number of DRB1 alleles transmitted to humans from the ancestral species.

It is inherent in the nature of maternal inheritance that all contemporary mtDNA lineages are derived from (or coalesce to) a single founding lineage. The hypothesis as stated by Cann *et al.* (*3*) simply postulated that this founding lineage was African and that the coalescence time was on the order of 100,000 to 200,000 years. The identification of a particular founding African mtDNA lineage says nothing about the size of the human population at that time. An estimate of the effective human population size (N_e), based on the diversity of mtDNA sequences among contemporary humans, was reported by Wilson and colleagues over a decade ago (*4*); $N_e = 6000$ females. Other recent estimates, based on classical polymorphisms (*5*) and Y chromosome–DNA markers (*6*), are also on the order of 10,000 individuals. Ayala argues that, when one considers various sources of error, these estimates are not inconsistent with his estimate of more than 100,000 individuals from the HLA data. In our view, however, a more realistic appraisal of the HLA class II sequence polymorphism also leads to an N_e of about 10,000, in line with N_e estimates from other molecular genetic data.

The extensive polymorphism at the HLA class II loci (for example, DRB1) is localized to the second exon, which encodes the peptide binding groove, and, in particular, to those codons encoding amino acids involved in interaction with the peptide and T cell receptor. The crux of the argument relating the contemporary HLA polymorphism to the size of the founding human population is the estimated number of alleles that were transmitted to the human lineage from the ancestral species. Obtain-

ing this estimate involves critical assumptions about the "age" of contemporary alleles and the rate of allelic diversification. Many DRB1 polymorphic sequence motifs are shared among different contemporary primate species, suggesting either conservation over millions of years of evolution, or convergent evolution. Many of the DRB1 "allelic lineages" (that is, a cluster of closely related alleles that may have a single serotype, such as DR1) may predate the separation of the hominoid species (5 to 7 million years ago). However, given the significant amount of postspeciation sequence diversification *within* these lineages inferred from phylogenetic tree analysis (*7*, *8*), the number of DRB1 alleles transmitted from the ancestral species to modern humans may be substantially less than that estimated by Ayala.

Ayala estimates that, of the 61 human DRB1 alleles included in the phylogenetic analysis [figure 3 of (*1*)], 31 are over 6 million years old; this number is arrived at by applying an estimate of the substitution rate ("molecular clock") to the phylogenetic tree. By now, there have been over 100 DRB1 alleles identified in all human populations, but most populations have fewer than 25 alleles. In general, HLA class II second exon sequences are not well suited to phylogenetic analysis and the construction of gene genealogies. Given the evidence for positive selection at sites encoding the antigen binding amino acid residues (*9*), as well as the patchwork pattern of polymorphism indicative of gene conversion-like events (*10*), the application of phylogenetic tree-building analyses to the polymorphic second exon DRB1 sequences is problematic, and interpretations based on the topology should be somewhat guarded.

In 1991, two of us showed that the phylogenetic tree for the first part of the second exon (encoding the β-sheet floor of the peptide binding groove) had a different topology from that of the second part, encoding the α-helical wall of the groove (*8*). Thus, different parts of the second exon of DRB1 have different evolutionary histories, and, hence, the length of the branches separating DRB1 alleles may not be proportional to time. Also, the use of molecular clock analyses, with the assumption of selective neutrality and a constant mutation rate, may be inappropriate for DRB1 second exon sequences and result in an inflated estimate of the divergence times and age.

The problem with applying a molecular clock to HLA polymorphism can be revealed by comparing intron and exon sequences of the DRB1 alleles (*11*). The sequence differences between alleles in different lineages are only slightly higher (about 1.5-fold) for the exon sequences than for the intron sequences. By contrast, the genetic distance among alleles within an allelic lineage is about 30-fold higher for the exon sequences than for the intron sequences, presumably due to selection. Applying an intron substitution rate for primate sequences (1.4×10^{-9} per site per year) (*12*) to these data indicates that the mean age of alleles within a lineage may be about 230,000 years, rather than the millions of years assumed in Ayala's calculation. Thus, these data suggest that only the allelic lineages predate the hominoid divergence, yielding an estimate of 8 to 9 rather than 31 DRB1 sequences that were transmitted to humans from the ancestral species.

With the use of a simulation model following populations containing 60 alleles over evolutionary time periods, Ayala concluded that, even with overdominant selection and selection coefficients (s) of 0.01 to 0.03, N_e must be greater than 100,000 to maintain the more than 30 presumed ancestral alleles. In general, how realistic are the assumptions in Ayala's model and how reliable is his estimate of N_e? On the basis of the data in our study (*11*) and on the considerations discussed above, the number of ancestral DRB1 sequences is likely to be less than 10 rather than over 30. Furthermore, this model assumes a selected mutation rate of 5×10^{-7} per gene per generation as the only mechanism for generating new alleles. Recent experimental evidence analyzing HLA class II germline variation in human sperm, potentially generated by interallelic gene conversion-like events, suggests a frequency of around 1/12,000 (*13*). Thus, even if only 10% of these new variants are selected for, the selected mutation rate would be more than an order of magnitude higher than the number assumed by Ayala. In isolated Native South Amerindian populations, previously unreported (and presumably newly arisen) class I and class II alleles have been identified (*14*), suggesting the generation of several new alleles within the last 10,000 to 20,000 years. In general, selective pressures other than heterozygote advantage may be operating, selecting rare newly arisen variants in different populations. Ayala cites the HLA-related resistance to malaria and plasmodium falciparum (*15*) as an example of heterozygote advantage; however, what was demonstrated in this study was protection conferred by particular DRB1 and HLA-B alleles, not the operation of heterozygote advantage in the Gambian population.

The Ayala paper [figure 7 in *1*)] shows that, even without considering a more realistic mutation rate, an N_e of only 10,000 is required to maintain 10 DRB1 alleles, the approximate number of ancestral DRB1 lineages inferred from the intron data (*11*). Contrary to Ayala's interpretation, the HLA DRB1 data are, in fact, consistent with the population sizes estimated by mtDNA sequence diversity and with the inference, based on mtDNA and recent CD4 haplotype data (*16*), of a population bottleneck associated with the "Out of Africa" migration. Thus, it is our view that the N_e of the early human population that can be estimated from the HLA polymorphism data is consistent with the estimates derived from the mtDNA sequence data that formed the basis for the "Mitochondrial Eve" hypothesis.

The distinction between $N_e > 100{,}000$ as opposed to $N_2 = 10{,}000$ is not trivial. It has been argued that the "Multiregional" evolution theory of human origins (*17*), which suggests that the transition from *Homo erectus* to *H. sapiens* involved population spread across the Old World from South Africa to Northern Europe and Asia, would require that N_e be much bigger than 10,000 (*18*). The fact that *all* of the genetic data—including mtDNA, Y chromosome–DNA markers, classical polymorphisms, and (as we argue here) HLA class II sequences—suggest that N_e is on the order of 10,000 provides one of the most compelling arguments against Multiregional evolution and in favor of a recent (African) origin of modern humans.

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Response: The "myth of Eve" I sought to dispel is the notion that evolutionists have shown that modern humans may have descended from one or a few women, a claim repeatedly made "in the media, as well as in popular scientific publications . . . and by scientists" (*1*, p. 1936). I cited Wesley M. Brown who, reporting mitochondrial DNA research done in Allan C. Wilson's laboratory, wrote (*2*, p. 3609)

> The amount of sequence heterogeneity observed, 0.18%, could have been generated from a single mating pair that existed 180 to 136 × 10^3 years ago, suggesting the possibility that present-day humans evolved from a small mitochondrially monomorphic population that existed at that time.

According to Erlich *et al.*, I estimated "that the size of the founding human population was at least 100,000." I did nothing of the kind. My core argument (*1*) was about the long-term effective size of human ancestral populations, which, I argued, must have been about 100,000 or more individuals in order to account for the extensive DRB1 polymorphism observed in current human populations.

I did, however, explore the matter of "population bottlenecks," such as may be traced to a founding population from which all modern humans might descend. I estimated that the minimum bottleneck size compatible with the DRB1 polymorphism would be 4490 to 4590 individuals, if the polymorphism were selectively neutral, and 4310 to 4380 individuals, if overdominant selection would be operating of magnitude $s = 0.01$, as I thought likely. I discussed other alternatives and averred (*1*, p. 1935)

> It may be concluded that, to account for the DRB1 polymorphism, the minimum possible number of individuals at a bottleneck is at least 4000; this number is consistent with the lower estimates derived from the mtDNA and the ZFY gene.

The conclusion by Erlich *et al.*, in their penultimate paragraph, is thus puzzling. That there is consistency between population estimates derived from HLA polymorphism data and those derived from mtDNA sequence data is not contrary to my interpretation, but rather what I explicitly asserted.

I shall briefly make two more points. One point refers to the statement by Erlich *et al.* that "molecular clock analyses, with the assumption of selective neutrality and a constant mutation rate, may be inappropriate . . . and result in an inflated estimate of the divergence times and age." I discarded the inflated estimates derived from the assumption of selective neutrality precisely because I assumed that the DRB1 second exon sequences are not selectively neutral. Moreover, the molecular clock analysis that I presented of the human gene lineages does not assume a constant mutation rate either; rather, it is based on an empirical assessment of the average rate of evolution of DRB1 alleles, obtained with the use of the "minimum" method by comparing many different species of known time of divergence (*3*).

The second point I wish to make is that my conclusion of the old age of the human DRB1 alleles was derived, first and foremost, from the distant association between some human gene lineages with each other, relative to their association with those from other primate species (*1*, figure 4 and p. 1931), and not from the molecular clock hypothesis (I explicitly noted that the gene lineages have evolved at different rates; see p. 1931).

> For example, the nine human genes at the top of Fig. 4 are closely related to one another, but they are more distantly related to other human genes in the genealogy than they are to the six genes immediately below them, including one drill gene and four macaque genes.

The divergence between the lineages that go one to humans and apes and the other to the Old World monkeys occurred 35 million years ago. It follows "that several human gene lineages already existed at that time" (*1*, p. 1931). The inference of a long-term population no smaller than 100,000 individuals is derived from the ancient age at which the gene lineages coalesce in one single ancestral gene. Thus, the arguments of Erlich *et al.* about how many lineages existed at one particular time and the age of particular subsets of lineages are not directly relevant to this inference.

The calculations of Erlich *et al.* about the age of introns assume that one accepts the molecular clock hypothesis and particular rates of substitutions, which are problematic. Moreover, as they point out, different parts of genes may have different evolutionary histories. The rates of divergence between introns and between exons may be disparate (*4*). Satta *et al.* (*5*) also have recently shown that the coalescence of DRB1 human alleles is "extraordinarily old"; the split of some allelic lineages occurred approximately 50 million years ago. The coalescence of human alleles of another gene of the HLA complex, the DQB1 locus, also occurred before the divergence between the Old World monkeys and the human lineage (*2*). Like the DRB1 data, the DQB1 results require $N_e \approx$ 100,000 individuals.

In their final paragraph Erlich *et al.* make two statements. One is that "*all* of the genetic data . . . suggest that N_e is on the order of 10,000." If by N_e they mean the long-term effective size of human populations, this is not correct (*1*). If they rather refer to the size of the founding population of modern humans, then the genetic data are *consistent* with such a statement. Second, Erlich *et al.* state that a founding population on the order of 10,000 individuals "provides one of the most compelling arguments against Multiregional evolution and in favor of a recent (African) origin of modern humans." Erlich *et al.* find the arguments in favor of a recent African origin to be compelling, but Templeton has recently stated that there is "no evidence that supports the hypothesis of an African–non-African population split either in the mtDNA or the nuclear DNA data" (*6*). It seems to me that Erlich *et al.*'s contention should be with Templeton, not with me. I responded to Templeton that "there is plenty of evidence" for the split, and stated my conviction that the weight of the evidence "favors a recent African origin for modern humans" (*7*). Nevertheless, I am not persuaded that the evidence currently available is compelling.

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