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Reply to Cordaux and Stoneking

To the Editor:

Cordaux and Stoneking (2003 [in this issue]) have argued that although there is evidence of anatomically modern humans (AMH) reaching Australia ~60,000 years ago (see also Bowler et al. 2003), there is no convincing genetic evidence that these early migrants passed through South Asia. They see this as obviating the need for a "southern migration" route for AMH out of Africa. They suggest that the ancestors of all known non-African mtDNA lineages (haplogroups M and N) entered Eurasia via the Levant ~45,000 years ago, prior to differentiating and dispersing to all land areas outside of Africa. Cordaux and Stoneking also seek to use estimated dates for the colonization of the Andaman Islands to lend support to their position.

The "crucial points" of evidence cited by Cordaux and Stoneking for their view on the settlement of South Asia are that "the South Asian mtDNA gene pool does not show close affinities to either Africa or PNG" (Papua New Guinea), and that "the archeological record does not show evidence for the presence of modern humans in South Asia before ~30,000 years ago." This position is inconsistent, since they first equate "modern" humans with Middle Paleolithic tools in Australia at ~50,000 years ago (Bowler et al. 2003) but later exclude South Asian settlers within the same time frame from being "modern" on the basis of their lithic industries. Here we re-evaluate the evidence for this position and show that the distributions and characteristics of mtDNA and Y-chromosome data actually accord better with a dispersal of AMH into South Asia from Africa >30,000 years ago. The same data also argue for the hypothesized "southern route" "out of Africa," leaving through Ethiopia via Arabia.

We agree with Cordaux and Stoneking that the diversity within Andamanese M2 is potentially useful for giving some idea of the settlement time for the Andaman Islands, because it is plausible that a regionally specific clade in the mtDNA genealogy (in this case, Andamanese M2) had its most recent common ancestor (MRCA) in that region. Of course, it is possible that related mtDNAs in the source population may have disappeared because of drift, but, in that case, the MRCA of the cluster in the settled region could either pre- or postdate the colonization event (for a careful discussion of the issues, see Richards et al. [2000]). An estimate of the time to the MRCA of the clade, with the available Andamanese data, provides an idea of the *latest* possible settlement time and not the earliest (contra Barbujani 1998 and Cordaux and Stoneking 2003 [in this issue]).

There are further statistical problems in the reply by Cordaux and Stoneking. In trying to justify a constant population size model for the Andamanese, they concentrate their calculations on a set of lineages *chosen to fall within a particular clade,* whereas the usual theory pertains to random sequences from the whole population, which, in the case of the Andamanese, contains the M4 clade. Thus, it is not clear what the sampling distributions of the test statistics under the null hypothesis would be for their approach. Therefore, it is not possible to assess the significance of the values computed. Further, their assertion that the point estimates of divergence are "almost certainly overestimated" because the varied sites are faster mutating than average is based on a misunderstanding of how mutations arise. The number of mutations accumulates proportionally to the *average* rate for the whole DNA stretch under consideration, irrespective of the individual site rates. Of course, some sites may be hit more than once, but this effect would lead to an *underestimation* of divergence times.

Cordaux and Stoneking introduce the finding by Thangaraj et al. (2003) that the present-day indigenous Andamanese belong to Y-chromosome haplogroup D, defined by the mutation M174. Although the date of haplogroup D is not incompatible with the Upper Paleolithic, they assume that both mtDNA and Y chromosome yield similar narratives regarding archeologically defined horizons. However, sex-specific processes may lead to more genetic drift in males than in females, so that the time depth of the Y genealogy would be more recent. Consequently, the apparently discordant Upper and Middle Paleolithic molecular dates, for Y and mtDNA, respectively, would not necessarily be incompatible.

There are other reasons that the estimated date to the MRCA of the M174 Y-chromosome clade (27,000–34,000 years) of Cordaux and Stoneking requires clarification. (i) The authors have not provided CIs for their estimate from a sample of just nine M174 individuals from two derived clades, and so the dates given only reflect uncertainty in the mutation rate. (ii) The Y SNP data sets used suffer from strong ascertainment bias (Underhill et al. 2000). Therefore, dating methods applicable to randomly selected mutations are likely to be seriously biased when applied to such data.

Using the subset of 42 unambiguous Andamanese mtDNA sequences available (Thangaraj et al. 2003) and considering the region 16090–16365 (Andrews et al. 1999) for which a plausible average mutation rate estimate is available (Forster et al. 1996), we estimate the time to the MRCA of the clade with motif 16223/16319/16357 as 33,000 years (SE 22,000) and to 16223/16319 as 53,000 years (SE 30,000). This method is based on the average number of mutations of each sequence from the ancestral one and an estimate of its SE (Saillard et al. 2000).

Given the large SEs of these estimates, the statement of Cordaux and Stoneking that this "suggests a colonization event…at most 40,000 years ago" cannot be sustained. In fact, the diversity of Andamanese M2 allows us to pin it down only to the last 100,000 years, but it must necessarily postdate the MRCA of haplogroup M, which is 65,000 years old (SE 7,000), on the basis of a recent dating from complete mtDNA sequences (Mishmar et al. 2003). The current estimate of coalescence for M2, on the basis of HVS-I variation, of 73,000 years (SE 22,900) does not further restrict its age (Kivisild et al. 2003).

Cordaux and Stoneking place their estimates of the first appearance of haplogroup M in South Asia within the context of a "southern route" of dispersal for AMH from Africa to Oceania. This hypothesis is directly linked to the absence of AMH in the Levantine corridor at the time of their putative presence in Australia ~60,000 years ago. On the other hand, Endicott et al. (2003) said "that haplogroup M may represent the phylogenetic signature of an early, southern colonization route in Asia." Neither the references for this statement nor the authors invoked an early settlement of Australia (contra Cordaux and Stoneking) to explain an African exodus leaving by a southern route (i.e., from Ethiopia via Arabia). Rather, it is the distribution and estimates of coalescence for mtDNA haplogroup M that are the basis for suggesting its early presence in South Asia.

All non-Africans have inherited a subset of African mtDNA haplogroup L3 lineages, differentiated into haplogroups M and N. The lack of other L3 lineages among all non-African mitochondria suggests that the earliest migration(s) of AMH already carried those two mtDNA ancestors or that they have replaced previously extant lineages. Haplogroup M is present in Ethiopia, but the rest of M is densely distributed throughout South and East Asia and is absent in the Near East and Europe. If the settlement of Asia by haplogroup M–bearing populations was delayed by the ~30,000 years alluded to by Cordaux and Stoneking, there is, as yet, no evidence of the source population, either inside or outside of Africa. Since haplogroup M is not present along their hypothesized “northern route” “out-of-Africa,” it seems likely to have been dispersed along the “southern route” and necessarily “early,” since the MRCA of Asian M is ~65,000 years old (Quintana-Murci et al. 1999; Stringer 2000; Mishmar et al. 2003).

The distributions, diversity, and ages of haplogroups M and N in India are consistent with this region providing the inocula for the subsequent differentiation of both the distinctive eastern and western Eurasian gene pools (Kivisild et al. 2003). This is because (i) the percentages of typically eastern and western Eurasian mtDNA lineages present in India are moderately low, (ii) the majority of these lineages are specific to India, and (iii) they contain all the founding haplotypes for the non-African mtDNA gene pool. The time to coalescence of the major M subclusters on the Indian subcontinent center on 47,000 years (SE 2,500 years) and not the ~30,000 years that Cordaux and Stoneking (2003 [in this issue]) allude to (Kivisild et al. 1999). These Indian components are comparable in diversity and are older than most eastern Asian and Papuan haplogroup M clusters (Forster et al. 2001). This suggests that the Indian subcontinent was settled soon after the African exodus and that there has been no complete extinction, or replacement, of the initial settlers (Kivisild et al. 2003). Of the Indian haplogroup M lineages, M2 is significant because of its estimated age (73,000 years, SE 20,000), southerly distribution, and frequency (~10% of caste populations and ~23% of noncaste populations) (Kivisild et al. 1999; Bamshad et al. 2001; Endicott et al. 2003).

The phylogeography of the four non-African Y-chromosomal founder haplogroups C, D, F, and K is also more consistent with the southern route migration than one through the Levantine corridor. This is because (i) the presence of the D lineage in the Andaman Islands completes the package of the postulated founder lineages detected in or around India, and (ii) C and D are spread, like mitochondrial haplogroup M, only in the Asian continent and Oceania (Underhill et al. 2001; Kivisild et al. 2003) and not in western Eurasia and North Africa. Further, the upper limit (contra Cordaux and Stoneking 2003 [in this issue]) for the time of their spread out of Africa would be the coalescent of all three main branches and not of only one of them, D-M174.

On the basis of the mtDNA and Y-chromosome data presented here, we see no need to accept the view of Cordaux and Stoneking regarding the settlement of South Asia. To suggest that in this region “the archeological record does not show evidence for the presence of modern humans…before ~30,000 years ago” is not sustainable if they accept their presence in Australia at ~60,000 years ago. This Eurocentric view of equating AMH with Upper Paleolithic tools has already been abandoned in the Middle East and Africa (McBrearty and Brooks 2000) and, given the Australasian evidence, should be discarded in South Asia, too. We must infer an early dispersal of AMH with non–Upper Paleolithic technology through Asia to explain the early Australian evidence (Stringer 2000), although we agree with Cordaux and Stoneking that the precise route(s) taken is still unclear (Stringer 2002). But we see no requirement for the South Asian mtDNA gene pool to demonstrate close affinities with either PNG or Africa to discuss an early settlement of this region. Given the continuity of the archeological record within India, from the Middle Paleolithic onward, and the range of estimated dates for Indian haplogroup M, there is no clear reason to preclude the presence of modern humans in this region prior to ~30,000 years ago.

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Single-Nucleotide Polymorphisms and Glaucoma Severity

To the Editor:

A recent publication in the *Journal* (Copin et al. 2002) reported that SNPs in the promoter of apolipoprotein E (APOE [MIM 107741]) modify the phenotype of primary open-angle glaucoma, result in increased optic-nerve damage, and interact at a highly significant level with an SNP in the promoter of myocilin (MYOC [MIM 601652]), a known glaucoma-causing gene. If correct, this would be of considerable importance for providing novel insight into the pathogenesis of a leading cause of worldwide blindness (Quigley et al. 1993), which is characterized by visual-field loss and progressive excavation (cupping) of the optic disc.

That study's conclusions are entirely dependent on the observation of differing disease severity in the genotypic subgroups. Glaucoma severity was graded by use of ordinal scales, and it is important to relate statistical analysis back to these scales. If we consider a simple example of patients with a bacterial infection that is scored (1, 2, or 3) according to whether they "got better," "stayed the same," or "got worse," if equal numbers got better and got worse, it would be meaningless to state that, on average, patients stayed the same (Campbell 2001). It would be equally invalid to present fractional differences in the data (e.g., 1.3). Unfortunately, Copin et al. (2002) employed this approach with both parameters used to gauge glaucoma severity.

The first parameter, cup-to-disc ratio (CDR), estimates in 10% (0.1) increments the proportion of the optic nerve that has been damaged. CDR is only an approximate guide because of high interindividual (normal range 0.0 to almost 0.9) and interobserver variability (>0.2) among specialists assessing optic discs (Lichter 1976; Jonas et al. 1988; Tielsch et al. 1988). Although CDR is a form of ordinal data with a fixed scale (0.1, 0.2, 0.3, etc.), Copin and coworkers (2002) report fractional differences (0.03 or 0.06), smaller than the scale increments, as evidence of increased disease severity with particular genotypes. The second parameter, visual-field loss, has been similarly evaluated. Recorded with an unspecified number of different techniques, the data were reanalyzed with a version