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Electronic-Database Information

The URL for data presented herein is as follows:

MitoKor, http://www.mitokor.com/science/560mtdnasrevision .php (for the revised 560 mtDNA coding-region sequences; "zip" and "sit" files also available)

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South Asia, the Andamanese, and the Genetic Evidence for an "Early" Human Dispersal out of Africa

To the Editor:

The out-of-Africa model of anatomically modern human evolution posits an African origin 100,000–200,000 years ago, followed by subsequent dispersal(s) to Eurasia and other continents within the last 100,000 years (Stringer and Andrews 1988). Although alternative models have been proposed, the out-of-Africa scenario receives the most support both from archeological and genetic evidence (Lahr and Foley 1994). However, the route(s) followed by the African migrants remain poorly understood. One proposed route was through northern Africa toward the Levant, which finds support in the archeological and fossil records (Lahr and Foley 1994). This exit of modern humans out of Africa would have taken place during the Upper Paleolithic era (∼45,000 years ago), which considerably postdates the earliest evidence of modern human presence in the Sahul. Indeed, luminescence dating, paleovegetation changes, and skeletal remains suggest that Australia was inhabited by modern humans by 60,000 years ago (Roberts and Jones 1994; Johnson et al. 1999; Miller et al. 1999; Thorne et al. 1999), implying a substantially earlier migration from Africa to Australia. To take this evidence into account, as well as morphological and archeological features of many Australian fossils, a second migration of modern humans, known as the "southern route" hypothesis, was suggested to have occurred during Middle Paleolithic times (60,000–100,000 years ago) from eastern Africa to Sahul via South Asia (Cavalli-Sforza et al. 1994; Lahr and Foley 1994).

In the January 2003 issue of the *Journal,* Endicott et al. (2003) investigated the genetic affinities of 11 Andaman islanders, a group of people in the Indian Ocean with phenotypic similarities to some African populations (i.e., "Negrito" features) and reputed to be possible descendants of early migrants out of Africa to Sahul, following the southern route. The authors claim that the results of their investigation "support the growing evidence of an early movement of humans through southern Asia." In our opinion, Endicott and colleagues' results do not support any relationship between the present Andamanese population and the hypothesized early southern migration. The authors identified three different mtDNA haplotypes in 11 Andaman islanders, two belonging to haplogroup M2 and one belonging to M4. These haplogroups had previously been reported only in the Indian subcontinent (Kivisild et al. 1999*b*; Bamshad et al. 2001). The Andaman M4 haplotype has been found previously in mainland India (Kivisild et al. 1999*b*), whereas the two Andaman M2 haplotypes are (so far) unique to the Andamanese. Given that (1) the latter two types occupy a basal position in the M2 network, which has an estimated coalescence time of 63,000 ± 6,000 years (Kivisild et al. 1999*b*), and (2) they are not found in mainland India, Endicott et al. (2003) conclude they represent an "early" settlement of the Andaman Islands. These two points need discussion.

Regarding point 1, the age of a haplogroup cannot be automatically equated to the age of subsets of this haplogroup. The founding type of haplogroup M2, characterized by 16223T and 16319A relative to the Cambridge reference sequence (CRS) (Anderson et al. 1981) (fig. 1),
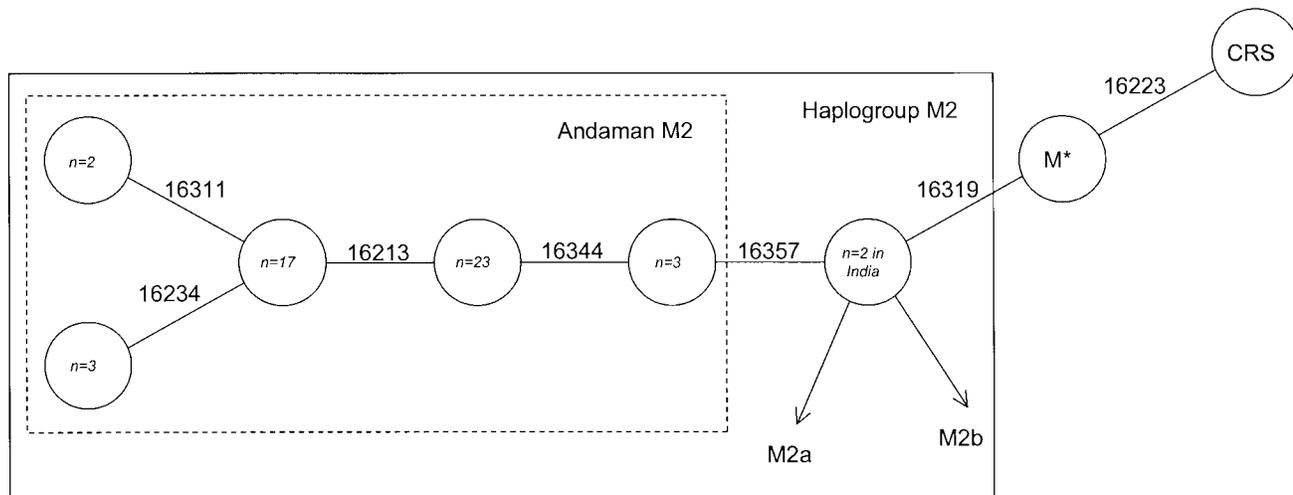
Figure 1 Median joining network (after Endicott et al. 2003) of mtDNA haplogroup M2. Substitution positions relative to the CRS are shown. The number of assigned individuals from sampling to date is indicated in the relevant nodes.

is dated to 63,000 years but is still present in mainland India. This does not mean that any cluster branching off of this node is 63,000 years old, but rather that it is, *at most,* 63,000 years old. In principle, the Andaman M2 cluster could be dated to any time between 63,000 years and today.

Regarding point 2, we do not question the fact that the Andaman M2 haplotypes have not been found in mainland India. However, related sequences are found in mainland India, one of which is cited by Endicott et al. (2003) as a candidate ancestral haplotype of the Andaman M2 sequences, since it differs by only one substitution at 16274 from one Andaman M2 haplotype. From figure 2 of Endicott et al. (2003), we identify in mainland India another candidate ancestral haplotype for the Andaman M2 types, namely, the founder type of the entire M2 haplogroup (characterized by 16223T, 16319A). This haplotype differs from one Andaman haplotype by a single substitution (at 16357) and has already been reported in mainland India (Kivisild et al. 1999*b*). Therefore, although identical haplotypes are not found elsewhere, the Andaman M2 haplotypes have closely related counterparts in present-day mainland India.

The extent of isolation experienced by Andaman islanders (and, thus, an approximate upper time limit for the colonization of the archipelago) can be estimated by dating the time needed to accumulate the observed variation of the Andaman-specific mtDNA lineages. In this respect, we analyzed 42 unambiguously determined mtDNA sequences from Onge, Jarawa, and Great Andamanese individuals (Thangaraj et al. 2003) and six sequences from Aka-Bea individuals (Endicott et al. 2003) belonging to haplogroup M2 (on the basis of the motif

16223T/16319A). Altogether, the 48 Andaman M2 sequences defined five haplotypes unified by 16357C (fig. 1). First, we investigated the pattern of demographic history for the data set of 48 Andaman M2 sequences, by calculating Fu's F_s (Fu 1997) and Tajima's D (Tajima 1989) (*P* values are based on 1,000 simulated samples), as well as Harpending's r (Harpending et al. 1993), as implemented in ARLEQUIN 2.0. Both Fu's F_s and Tajima's D were not significantly different from zero ($F_s = -0.66$, $P = .35$; $D = -0.19$, $P = .50$), suggesting no demographic expansion of the Andamanese population. In addition, Harpending's r value was 0.146, consistent with a scenario of constant population size.

Assuming a constant population size, the Kimura two-parameter model of substitution (as implemented in MEGA 2.1) and a mutation rate of 1.65×10^{-7} per site per year (corresponding to 33% divergence per million years [Ward et al. 1991]), divergence times of the Andaman M2 haplotypes relative to two different founders were estimated. When the most ancestral Andaman-specific M2 type (bearing the motif 16223T, 16319A, 16357C) (fig. 1) was used as a founder, the average time needed to accumulate the observed variation in Andaman Islands was 25,300 years. When the Indian candidate as ancestral type of the Andaman M2 sequences (bearing the motif 16223T, 16319A) (fig. 1) was used as a founder, the average time back to the Indian common ancestor was 42,300 years. These results indicate that Andaman islanders may have remained isolated from mainland India for 25,000–42,000 years. However, this time is almost certainly overestimated. The average substitution rate of the five substitutions found in the Andaman M2 cluster (16213, 16234, 16311, 16344, 16357)

(fig. 1) is ~2.5 times that of the control region as a whole (Meyer et al. 1999); thus, the real time to accumulate the observed variation may be up to 2.5-fold less, or 10,100–16,900 years. Moreover, the time estimates are based on the assumption that all the Andaman M2 types are derived from a single founding lineage; more recent times would result if there was more than one founding lineage. In summary, the presence of the unique M2 lineages in the Andaman Islands (as well as the Andaman M4 lineage that is identical to present-day Indian M4 lineages) suggests a colonization event from the Indian subcontinent occurring during the Upper Paleolithic (or perhaps even more recently), at most 40,000 years ago, which is still at least 20,000 years after the early southern-route migration to Sahul.

Thangaraj et al. (2003) also presented Y-chromosome evidence for Andaman islanders. They suggested that haplogroup D, an Asian-specific YAP lineage bearing the derived state at M174 (Underhill et al. 2000), is an original paternal lineage of Andaman islanders. To estimate the age of M174, we calculated that the average number of mutations from M174 to contemporary haplotypes was 4.89, on the basis of the data in Underhill et al. (2000). Underhill et al. (2000) estimated that the average time for a new mutation was 5,600–6,900 years, which puts the age of M174 at 27,000–34,000 years. Since the age of M174 is an upper estimate for the time when this Y haplogroup reached the Andaman Islands, the Y-chromosome evidence suggests an upper figure of ~30,000 years for the colonization of Andaman Islands, in good agreement with the mtDNA evidence. Hence, both Y-chromosome and mtDNA data support an Upper Paleolithic rather than Middle Paleolithic settlement of the archipelago.

Other studies have shown that mtDNA haplogroups M and U are common in the Indian subcontinent, and both have coalescence times of 50,000–70,000 years (Quintana-Murci et al. 1999; Kivisild et al. 1999a, 1999b, 2003). This has been taken as a genetic signature for an "early" (i.e., Middle Paleolithic) colonization of South Asia by modern humans and, consequently, as a confirmation of the "southern route" hypothesis. However, in South Asia, the earliest evidence of modern human industries and remains is dated to ~30,000 years (Kennedy and Deraniyagala 1989; Misra 2001). Because earlier records from Neanderthal-like technologies are found in South Asia (Misra 2001), the absence of modern human traces does not seem to be due to a gap in the archeological record. How to explain a gap of at least 20,000 years long between the archeological and genetic evidence? The coalescence time of haplogroup U in Europe is also dated to 50,000–60,000 years (Richards et al. 2000), but the accepted time scale of the peopling of Europe by modern humans is 35,000–40,000 years ago (Bocquet-Appel and Demars 2000). If so, why should

South Asia, but not Europe, be considered to have been colonized 50,000–70,000 years ago? The observed timing discrepancies between genetic and archeological estimates are simply explained by the fact that the divergence of genes predates that of populations (Barbujani et al. 1998). Consequently, the evidence for a Middle Paleolithic colonization of the Indian subcontinent provided by mtDNA studies and the genetic confirmation of the "southern route" hypothesis are all the more debatable.

What conclusive genetic evidence remains for the "southern route" hypothesis? If the Indian subcontinent had received migrants from this putative migration from Africa to the Sahul, it would be reasonable to expect at least some remote affinities between African, Indian, Australian, and/or Papua-New-Guinean (PNG) gene pools. Unfortunately, to date, most studies aimed at detecting traces of an ancient peopling of South Asia through the southern route have not compared the Indian data in a wider context, in particular with people (except Africans) from regions believed to have been involved in the "southern route" migration. *Alu* insertions data are interpreted as supporting an ancient African-PNG relationship, but India is not a part of this relationship (Stoneking et al. 1997). Y-chromosome and mtDNA data suggest a connection between the Indian subcontinent and Australia, which is, however, dated to <5,000 years (Redd and Stoneking 1999; Redd et al. 2002; Cordaux et al. 2003). Thangaraj et al. (2003) suggest a possible common origin for the inhabitants of the Andaman Islands and PNG, on the basis of high frequencies of mtDNA 16357C in these two areas only. However, we note that 16357C in Andamanese mtDNAs occurs on the background of 16223T and 16319A, corresponding to haplogroup M (Endicott et al. 2003; Thangaraj et al. 2003). In contrast, 16357C in PNG is associated with 16223C and 16319G (Hagelberg et al. 1999; Redd and Stoneking 1999), corresponding to haplogroup N. Consequently, the presence of 16357C in the Andaman Islands and PNG is most likely the result of parallel mutations and not a signature of shared ancestry. In addition, the putative original paternal lineage of Andaman islanders (i.e., Y haplogroup D) (Thangaraj et al. 2003) is not found in Melanesia or Australia (Kayser et al. 2003). Otherwise, mtDNA haplotypes in South Asian ethnic groups are most closely related to east Eurasians and do not show any particular ties to African or PNG populations (Kivisild et al. 2003; Cordaux et al. 2003). In addition, an mtDNA control region motif proposed by Forster et al. (2001) to represent a signature of an early migration from Africa to Sahul through the southern route is not found in South Asia (Cordaux et al. 2003). In summary, there is no convincing support to date for a Middle Paleolithic genetic contribution to

South Asia by migrants from Africa to Sahul along the southern route.

If so, and in light of the genetic and archeological evidence, the most reasonable scenario for the peopling of South Asia is an Upper Paleolithic event (i.e., the major expansion of modern humans out of Africa through the Levant [Lahr and Foley 1994]), from which the current Indian gene pool is derived. Proto-Eurasians subsequently evolved to their present distinct South Asian, East Asian, and European gene pools and expanded ~30,000 years ago (Forster et al. 2001). Without requiring a Middle Paleolithic migration of modern humans into South Asia, this scenario explains why (i) most South Asian mtDNA clusters coalesce and show signs of demographic expansions ~30,000 years ago (Kivisild et al. 1999b), (ii) the South Asian mtDNA gene pool is related to (but distinct from) other Eurasian mtDNA pools, (iii) the South Asian mtDNA gene pool does not show close affinities to either Africa or PNG, and (iv) the archeological record does not show evidence for the presence of modern humans in South Asia before ~30,000 years ago. Hypothesizing a Middle Paleolithic migration to South Asia would create more problems than it would solve: it would, in particular, hardly explain the above crucial points iii and iv.

We conclude that there is currently no convincing genetic evidence that supports the postulated Middle Paleolithic migration of modern humans from Africa to the Sahul through South Asia. This does not necessarily mean that such a migration never occurred, since archaeological evidence does document modern humans in Sahul by ~60,000 years ago. However, it is possible that subsequent Upper Paleolithic migrations in Eurasia erased the genetic traces in contemporary populations of this early event in our history (Cavalli-Sforza et al. 1994; Cordaux et al. 2003); in any event, the "southern route" hypothesis still awaits genetic support.

Acknowledgments

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Electronic-Database Information

URLs for data presented herein are as follows:

ARLEQUIN 2.0, http://lgb.unige.ch/arlequin/
MEGA 2.1, http://www.megasoftware.net/

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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