L, Savontaus ML (1996) The genetic relationship between the Finns and the Finnish Saami (Lapps): analysis of nuclear DNA and mtDNA. Am J Hum Genet 58:1309–1322
Menozzi P, Piazza A, Cavalli-Sforza LL (1978) Synthetic maps of human gene frequencies in Europeans. Science 201: 786–792
Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York
Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. Evolution 29: 1–10
Piazza A, Rendine S, Minch E, Menozzi P, Mountain J, Cavalli-Sforza LL (1995) Genetics and the origin of European languages. Proc Natl Acad Sci USA 92:5836–5840
Rendine S, Piazza A, Cavalli-Sforza LL (1986) Simulation and separation by principal components of multiple demic expansions in Europe. Am Nat 128:681–706
Renfrew C (1987) Archaeology and language: the puzzle of Indo-European origins. Jonathan Cape, London
———(1991) Before Babel: speculations on the origins of linguistic diversity. Camb Archaeol J 1:3–23
Richards M, Côrte-Real H, Forster P, Macaulay V, Wilkinson-Herbots H, Demaine A, Papiha S, et al (1996) Paleolithic and Neolithic lineages in the European mitochondrial gene pool. Am J Hum Genet 59:185–203
Richards M, Macaulay V, Sykes B, Pettit P, Forster P, Hedges R, Bandelt HJ (1997) Reply to Cavalli-Sforza and Minch. Am J Hum Genet 61:251–254
Semino O, Passarino G, Brega A, Fellous M, Santachiara-Benerecetti AS (1996) A view of the Neolithic demic diffusion in Europe through two Y chromosome–specific markers. Am J Hum Genet 59:964–968
Sokal RR, Harding RM, Oden NL (1989) Spatial patterns of human gene frequencies in Europe. Am J Phys Anthropol 80:267–294.
Sokal RR, Oden NL, Wilson C (1991) New genetic evidence for the spread of agriculture in Europe by demic diffusion. Nature 351:143–145
Stenico M, Nigro L, Barbujani G (1998) Mitochondrial lineages in Ladin-speaking communities of the eastern Alps. Proc R Soc Lond B Biol Sci 265:1–7
Tajima F (1983) Evolutionary relationships of DNA sequences in finite populations. Genetics 105:437–460

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Reply to Barbujani et al.

To the Editor:

We agree entirely with Barbujani et al. (1998 [in this issue]) that the age of a group of haplotypes cannot be mechanically equated to the age of the population from which they come and that such an uncritical equation would artificially elevate the estimated age of the population under study. However, our analysis (Richards et al. 1996, p. 194) focuses not simply on haplogroups, but on haplotypes within haplogroups. Such an analysis depends critically on the correct identification—by cross-population comparison of lineages—of all of the major founder haplotypes, which can then be used as a baseline from which to date the founder events associated with each cluster of haplotypes. This is exemplified in our paper by the identification of a number of distinct founder haplotypes in lineage group 2A, picked out on the basis of their presence as shared ancestral nodes in the European and Near Eastern phylogenies, which root deeply (during the Upper Paleolithic period) in the Near Eastern data but which have accumulated only a small amount of variation—equivalent to ~10,000 years or so—within Europe. This suggests, to us, expansion into Europe from the Near East during the Neolithic period. Of the various lineage clusters that we identified in Europe and the Near East, only group 2A showed this pattern; other clusters did not show evidence of recent founder events within Europe.

We believe that a phylogeographic analysis such as this—which is indeed based on molecules rather than on populations—is capable of a much finer resolution than one based on distance statistics, such as that suggested by Barbujani et al. (1998). Moreover, the particular statistic used is misleading, as it is based on a model of populations of constant size at mutation-drift equilibrium, which is patently unsuitable for application to Europe and the Near East. However, an important weakness of our published analysis is the meager volume of comparative data from the Near East: essentially 42 individuals, mostly from the Arabian peninsula. In subsequent work, we are extending the analysis to a much larger sample from southwestern Asia, to improve our confidence that most founder haplotypes have been identified. We aim to report our conclusions in the near future.

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References

Barbujani G, Bertorelle G, Chikhi L (1998) Evidence for Paleolithic and Neolithic gene flow in Europe. Am J Hum Genet 62:000–000 (in this issue)
Richards M, Côrte-Real H, Forster P, Macaulay V, Wilkinson-

Herbots H, Demaine A, Papiha S, et al (1996) Paleolithic and Neolithic lineages in the European mitochondrial gene pool. Am J Hum Genet 59:185–203

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Reply to Hofmann et al.

To the Editor:

In the June issue of the *Journal*, Hofmann et al. (1997) commented on our study of Leber hereditary optic neuropathy (LHON; MIM 535000 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?535000]) multigeneration pedigrees (Mackey et al. 1996). Hofmann et al. made two main points in their letter, the first of which was that a mutation at nucleotide 15257 of the mitochondrial cytochrome *b* gene has a pathogenic role in LHON. This is a long-standing and unresolved controversy that epitomizes the pitfalls that beset the identification of pathogenic mtDNA mutations. Their second point, which is not an issue that we addressed, was that "so-called" secondary LHON mutations, in their terminology, play an etiologic role in other neurological disorders.

There is broad agreement that mtDNA mutations at nucleotides 3460, 11778, and 14484 are pathogenic LHON mutations, but there is disagreement over the pathogenic role of the 15257 mutation (Howell 1994, 1997*a*; Oostra et al. 1994). In our previous reports, including that by Mackey et al. (1996), the term "primary" was applied to the 3460, 11778, and 14484 LHON mutations. LHON is maternally inherited, but the penetrance is incomplete. The pathogenic mtDNA mutation is thus the predominant risk factor, but additional etiologic factors are required for manifestation of the optic neuropathy (Howell et al. 1997*a,* 1997*b*). In that sense, the 3460, 11778, and 14484 mtDNA mutations have a *primary* pathogenic role in LHON. Several additional mtDNA mutations have been identified that may have an etiologic or pathogenic role in LHON, including some that may augment or modify the phenotypic effects of the three known pathogenic mutations (see below). As a result of this uncertainty, the nomenclature has become commensurately more complicated. For example, Brown and Wallace (1994) list 16 mutations that have primary, secondary, or intermediate roles in LHON, although even this list is now incomplete (e.g., see Howell et al. 1998, and references therein).

The purpose of our previous study (Mackey et al. 1996) was to identify the pathogenic mtDNA mutations in LHON pedigrees. A total of 159 families (comprising ~12,000 maternal relatives) from Australia and northern Europe were analyzed, because these are countries where extensive genealogies are more easily obtained. We limited our study to large multigeneration LHON families, to avoid the ambiguities that arise with singleton cases of bilateral optic neuropathy in which maternal inheritance is lacking. The majority of sporadic cases of a LHON-like optic atrophy are not associated with the 3460, 11778, or 14484 LHON mutations (Chan et al. 1996). *None* of these 159 LHON families carried the 15257 mutation in the absence of one of the three previously established LHON mutations, although it was associated with one of the three mutations in six LHON families. This association has also been found in other LHON families (Howell et al. 1993; Oostra et al. 1994), as well as among those analyzed by Hofmann et al. (1997). The penetrance of pathogenic mutations is not increased in the LHON families whose mtDNAs also harbor the 15257 mutation (e.g., see Howell et al. 1993; Torroni et al. 1997). These negative results argue against a pathogenic role for the 15257 mutation, because LHON is a disorder whose penetrance is particularly dependent on the action of secondary etiologic factors (Howell 1997*a,* 1997*b*).

In contrast to our results, Obermaier-Kusser et al. (1994) reported a LHON family, with multiple affected family members that span multiple generations, that carries the 15257 mutation but not one of the three previously identified pathogenic LHON mutations. Hofmann et al. (1997) report a total of 55 optic neuropathy index cases, 3 of which are 15257 plus 11778 and 6 of which are 15257 plus 14484, but 5 of which are "15257 only." It is not clear whether these 15257-only cases are distant relatives of the LHON family described elsewhere (Obermaier-Kusser et al. 1994) or have affected maternal relatives. It is precisely because of the experimental and analytical difficulties inherent to singleton cases that we undertook our study of multigeneration families. More important, sequencing analysis has not been performed, either for the 15257-only LHON family or for the new 15257 cases, and the presence of a rare, unidentified pathogenic mutation cannot be ruled out.

The 15257 mutation has been detected at a low frequency (Brown et al. 1992; Kalman et al. 1995; and especially see Torroni et al. 1997) in normal control subjects, a result that argues against a pathogenic role. However, population surveys of normal controls should capture individuals who harbor a pathogenic LHON mutation but who, because of the incomplete penetrance, are not clinically affected. The 3460, 11778, and