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Reply to Cavalli-Sforza and Minch

To the Editor:

In a recent paper (Richards et al. 1996), we used a phylogeographic approach to infer that most (>85%) of the mtDNA control region (D-loop) variation in present-day Europeans has an ancient ancestry within Europe, coalescing during the Upper Paleolithic. This seems to be in contrast with earlier principal-component analyses of nuclear-gene frequencies in Europe, widely interpreted as evidence for a substantial Neolithic settlement from southwest Asia, which overwhelmed the Mesolithic hunter-gatherers. This apparent conflict has engendered the response by Cavalli-Sforza and Minch. They make criticisms of our treatment of the data in particular and of the reliability of mitochondrial control-region sequences in general, both of which criticisms we will address below. It is worth noting at the outset, however, their new suggestion that the proportion of the variation accounted for by the first principal component (26%) is "probably not very far" from the proportion of genes contributed by Neolithic newcomers to the European gene pool. Were this correct, it might seem that there could be little room for debate, since we could agree that the genetic contribution of the newcomers, while not insignificant, was relatively minor. However, there is more to the issue than this.

With regard first to their specific criticisms of our paper, it is precisely because there is little of interest to be learned from population-based comparisons using a single locus that we adopted a genealogical approach. There was—and apparently still is—a basic misunderstanding concerning the way in which mtDNA and Y-chromosome sequences should be analyzed for population studies. Traditionally, nuclear-allele frequency data have been the target of investigation, but, because recombination operates on such data in every generation, such analyses are inevitably restricted to coarse-grained summary statistics at the population level (diversity measures, population trees, principal-component maps, etc.). The resulting loss of information is then compensated in part by taking a large number of such loci into consideration. With mtDNA (or, for that matter, any other single locus), this approach is bound to be rather uninformative, and it is no surprise that earlier reports of European mtDNA diversity (Pult et al. 1994; Bertranpetit et al. 1995) were unable to detect significant structure. Table 2 in our earlier paper testifies to the futility of applying diversity measures between populations to mtDNA. We evidently did not emphasize this clearly enough in our paper, leading Cavalli-Sforza and Minch to miss our point and to reiterate this unhelpful test scenario by use of table 4 in our previous paper.

Our own approach is very different. It has been argued within evolutionary biology, for at least a decade, that mtDNA is "not just another molecular marker" (Avise et al. 1987, p. 516), in that it offers the currently unrivaled opportunity for estimation of intraspecific phylogenies (both gene trees and, potentially, coalescent trees) and to detect geographical patterns in distributions and ages of clusters or clades. This approach was promoted by Allan Wilson and his colleagues (e.g., see Cann et al. 1987) and has been aptly termed "phylogeography" by Avise et al. (1987). The work of Templeton et al. (1995 [and earlier references therein]), our work (Bandelt et al. 1995; Forster et al. 1996; Richards et al. 1996), and the work of several others (Torroni et al. 1996 [and references therein]; Ward and Valencia 1996 [and references therein]; Harding et al. 1997) can be situated within this phylogeographic tradition.

A phylogenetic analysis also allows one to evaluate the information content of different stretches and positions in the mitochondrial genome, relative to an envisaged time depth. Although Cavalli-Sforza and Minch dismiss the possibility that mtDNA sequence variation contains much genuine information, a high level of noise would in fact have easily been detected by our phylogenetic-network method. It is, for instance, quite evident that the second hypervariable segment of the control region offers little phylogenetic information in the case of European sequences, since a handful of positions appear to be extreme mutational hot spots (Aris-Brosou and Excoffier 1996; Torroni et al. 1996). By contrast, control-region sequences from the first segment, especially when additionally supported by restriction-site data from the entire mitochondrial genome, are highly informative—at least within a time scale of the past 100,000 years or so. Cavalli-Sforza and Minch thus confound a perceived inadequacy of the mitochondrial control region with the genuine futility of applying certain analytical tools (such as population trees, principal component maps, and so on) to mtDNA for reconstruction of historical processes of human dispersal. Their attempts to explain the seeming homogeneity of European mtDNA therefore lead to ad hoc explanations about the nature of mtDNA variation in human populations, such as when "heteroplasmy," "high female migration," and "hypergamy" are invoked to postulate a depression of genetic distances in non-Africans. Yet, all the evidence to date for humans (Bendall et al. 1996; Howell et al. 1996; Parsons et al. 1997) points to a relatively rapid fixation mechanism for new mitochondrial mutations, so that the intermediate state of heteroplasmy does not persist for more than a few generations. As for different mating patterns between the sexes, we have always been clear that maternal-lineage reconstruction using mtDNA has nothing direct to say about the contribution of males to the gene pool. Parallel studies using Y-chromosome variation will be more helpful in addressing these issues than are assumptions about the unknown reproductive behavior of prehistoric populations.

The differences between ourselves and Cavalli-Sforza and his colleagues should not allow us to miss what we have in common. We can agree that pure indigenism is mistaken and that the Neolithic newcomers left a definite mark in the European gene pool, albeit as a minority. This is suggested by both mitochondrial and Y-specific markers (Semino et al. 1996) and no longer hinges entirely on a particular interpretation of synthetic maps for nuclear frequency data. Indeed, the synthetic maps for Europe that have been provided by Cavalli-Sforza et al. (1994) are not incompatible with the scenario outlined in our paper. However, there is no good reason for believing that the first principal component in Europe is due entirely to Neolithic rather than to, say, early Upper Paleolithic settlement or even a mixture of the two. It is hard to see why principal components and migrations (or demic diffusion processes) should correspond to each other in a one-to-one fashion. Two or more migrations may contribute to a single component, and it may well be that the first principal component in Europe is a palimpsest of early Upper Paleolithic, Neolithic, and perhaps other expansions from southwest Asia. In this context, there is an interesting qualitative similarity between the radiocarbon map for the spread of the Neolithic (e.g., see fig. 1 of Cavalli-Sforza and Minch) and that for settlement in the early Upper Paleolithic (see fig. 1). Finally, there is no good reason either for the suggestion of Cavalli-Sforza and Minch that the variation accounted for by the first principal component ($\sim$26%) is directly related to the proportion of the European gene pool contributed by the newcomers, because the extent of variation described by the first principal component depends not only on the number of incoming settlers but also on their genetic distance from the native population—and even on *subsequent* population movements.

Contrary to some of the most detailed considerations of the archaeological evidence in recent years (e.g., see Whittle 1996), the mtDNA data suggest that new colonization of Europe from southwest Asia did indeed occur during the Neolithic, as Cavalli-Sforza and his colleagues proposed (Menozzi et al. 1978; Ammerman and Cavalli-Sforza 1984). Nevertheless, it seems likely that their interpretation underestimates the Mesolithic contribution. Furthermore, the model of demic diffusion by means of a wave of advance may also give the wrong impression about the nature of Neolithic colonization. Archaeologists have pointed out that the radiocarbon maps on which the model was originally based were constructed on the assumption of a uniform "Neolithic package." This does not hold true for most of the maps' sites outside

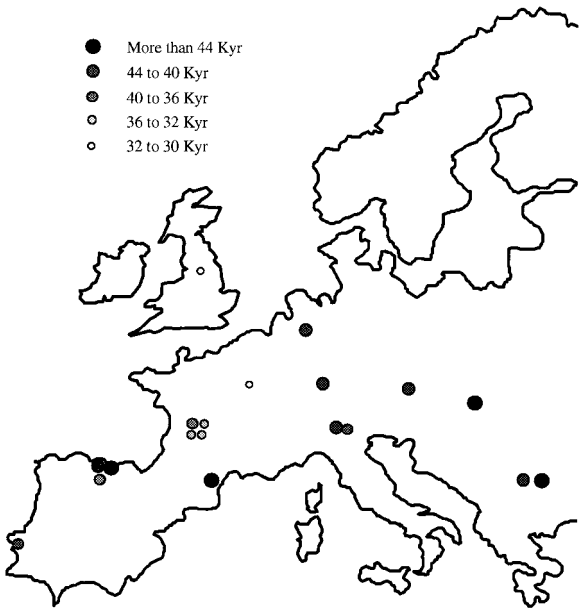

Figure 1 Radiocarbon evidence for the spread of the early Upper Paleolithic in Europe, including all assemblages with radiocarbon dates earlier than 30,000 years before the present, regardless of their specific taxomic facies. Dates were taken from the publications listed below and from a database assembled by S. W. G. Davies. The chronological data support an east-west movement (Djindjian 1991; Koslowski 1992; Mellars 1992; Hahn 1993). The earliest dates are from the Balkans and the middle Danube basin (Haesaerts 1990; Koslowski 1992). Early dates in the Mediterranean (Bartolomei 1992; Bischoff et al. 1989; Straus 1994) suggest an initial, rapid spread of the Aurignacian, restricted to the northern Mediterranean coast, although isolated examples of early dates in Germany and Belgium may also indicate an early expansion up the Rhine (Hahn 1993; Otte 1990) by way of extension from movement along the Danube corridor (Koslowski 1992). The two routes of expansion are strikingly similar to the pattern of the later spread of the Neolithic.

the central LBK (*Linienbandkeramik*) zone, and when this is taken into account the rate of spread is more punctuated (Zvelebil 1989; Thomas 1996). This receives some support from the mitochondrial data, in which the probable Neolithic lineages occur typically in only 10%–15% of total lineages and do not show southeast-northwest clines. Taken together with the presence of geographically specific lineages within the Neolithic cluster, this suggests a rapid penetration from the southeast, both westward and northwestward, followed by a much more gradual intermixing with the numerically dominant indigenous Mesolithic inhabitants. This view is also in line with paleoecological evidence (Willis and Bennett 1994), which shows no immediate environmental impact associated with the earliest radiocarbon dates for the European Neolithic.

Referring to the demic and cultural components of the Mesolithic-Neolithic transition, Ammerman (1989, p. 163) has suggested that "the real challenge for the archaeologist must be to evaluate the relative importance of the two different modes in various regions of Europe." Classical genetic analyses have given us limited purchase on this problem. The phylogeographic approach, pioneered with the use of mtDNA and increasingly being applied to the Y chromosome and other nuclear loci, seems to us a more promising route to take.

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Nonparametric Linkage Tests Are Model Free

To the Editor:

I followed with interest the recent exchange between Greenberg et al. (1996, 1997) and Farrall (1997). A major point of contention is whether affected-sib-pair (ASP) and other nonparametric linkage tests are really model free. Greenberg et al. (1997) contend that, because such tests are equivalent to LOD score tests under particular genetic models, they in fact implicitly assume a model and are "hardly model free in the usual understanding of that phrase." Farrall argues that the tests are nonparametric in the sense that they do not require that a particular model be specified a priori. Who is right?

The argument can be settled by referring to standard statistical literature on nonparametric statistics. Lindgren (1968, p. 400) defines "distribution free" (or "model free," or "nonparametric") tests as "procedures involving a statistic whose distribution (at least