

PREFACE

The Y Chromosome Consortium (YCC) is a group involved in a collaborative effort to study genetic variation on the human Y chromosome. The approach is the following: i) to establish a repository of lymphoblastoid cell lines (the Repository) derived from a sample of individuals representing indigenous populations from all inhabited continents, ii) to provide DNA isolated from these cell lines to investigators searching for polymorphisms on the Y chromosome, and iii) to establish a common database (the Database), in which will be pooled the results of typing DNAs from the Repository cell lines at as many Y-specific polymorphic loci as possible. The YCC will function in a similar fashion to the CEPH international collaboration.

The YCC Newsletter is a forum for communicating progress in the field of evolution of the human Y chromosome. In particular, this Newsletter will contain a regular update of the YCC Repository, the YCC Database and other information (polymorphisms, participants, recent publications, meetings, etc.). The YCC Newsletter will be published twice a year. The Editors welcome contributions to the YCC Newsletter. In preparation for the symposium at the next annual meeting of the American Society of Human Genetics (see below), so that the proceedings there may be recorded in the Newsletter, the next publication deadline is October 1, 1995.

SYMPOSIUM

At the 1993 annual meeting of the American Society of Human Genetics (ASHG), we (Michael Hammer and Nathan Ellis) submitted a proposal to the Program Committee for a workshop on using Y chromosomal variation to study the origin and migration patterns of modern human populations. The Program Committee did not include the workshop in last year's meeting; however, the proposal was considered again this year as a symposium. Based on the suggestions of the Program Committee contact, Aravinda Chakravarti, we revised the proposal to include more data on other components of the human genome. Our proposal entitled **Modern human origins: Perspectives from Y**

chromosomal, mitochondrial, and autosomal DNA studies, was selected by the Program Committee and will be part of the scheduled program at the 1995 annual meeting to be held in October in Minneapolis.

For ASHG symposia, there is a limit of a maximum of five speakers, two of which could receive funding for travel. With these constraints, the organizers had the difficult job of selecting just five speakers to represent all of the major topics (i.e., the human Y chromosome, mitochondrial, and autosomal DNAs). By necessity, then, criteria were needed for selection. First, we decided that preference should be given to younger investigators who have not had the opportunity to speak at major meetings. Secondly, preference was given to speakers with polymorphism data from several different human populations or to those who could synthesize information from data sets of different types of DNA (Y chromosomal, mitochondrial, and autosomal). Still, the final decision was very difficult because many excellent people responded to our queries by sending abstracts, and they offered to speak at the symposium. We warmly thank all the respondents. The following (in order of oration) are the five selected speakers: Mark Stoneking ("MtDNA and modern human origins"), Rosalind Harding ("Gene trees for β -globin sequences: inferences on the evolution of modern humans"), Chris Tyler-Smith ("The use of Y chromosome DNA polymorphisms to study human evolution"), Michael Hammer ("The origins of Y chromosome sequence diversity"), and Lynn Jorde ("Origins and affinities of modern humans: a comparison of mitochondrial and nuclear polymorphisms"). With such excellent and imaginative speakers, we look forward to a thought-provoking and exciting symposium.

Roundtable Discussion

Announcing the *Third Roundtable Discussion on the Evolution of the Y Chromosome* to take place at the 1995 annual ASHG meeting. The Roundtable Discussion will 1) provide an open forum for the discussion of Y chromosomal variation and the use of the Y chromosome in the study of human evolution, 2) provide the opportunity for those who were not

included in the symposium to present their results, 3) widen the activities of the YCC by encouraging new interactions between laboratories and 4) serve to advance the goals (oft stated in the pages of this Newsletter) of the YCC. The Roundtable Discussion will be held on one of the open evenings during the ASHG meeting in Minneapolis (October 1995), and it will be followed immediately by the *Fourth International Meeting of the YCC Ad Hoc Committee* to conduct official business of the YCC. Please see the schedule published by the American Society of Human Genetics.

Come one and all; come relax and unzip in the dimly lit somnolent enclosures of the Minneapolis Convention Center's conference rooms.

REPOSITORY UPDATE

Hurrah! Action in the Repository

The Repository contains now 64 accessioned cell lines (three of which were lost following accession) with the possibility of introducing 9 more cell lines from resources in stock. In addition, six new cell lines have been purchased from the Coriell Human Diversity Panel (see Appendix 1). These 76 cell lines bring us to the mid-way point in our collection, the goal being to have 150 cell lines in the Repository. Because accessioning of new cell lines has slowed tremendously in the last year, we decided to give the process a boost (we hope) by preparing and distributing DNA from this first group of cells.

The Elliad. Sing Goddess of the wrath of the cultures, that baneful wrath that sent so many strong cells to dark death deep entubed to be the feast of dogs and birds, when first the terrible conflict began between manly Hammer of the big hairy pilluck and the humble and modest Ellis son of Francis. Now when the academic men on both sides were set in order, the haughty Hammer 'came on with clamor and shouting, like wildfowl, as when the clamor of cranes goes high to the heavens', and, 'as the multitudinous nations of birds winged,' he flew into New York City over the 1994 Thanksgiving Vacation to do baleful battle against the dread cell-cultures whose hereditary material his angry heart sought for the YCC Repository. At daybreak, the

humble and modest Ellis 'went silently, breathing valor, stubbornly minded in his heart to stand by the other.'

Warlike Hammer leapt from the ranks as challenger, wearing across his shoulder the hide of a leopard,' he challenged all the best of the cell lines to fight man to cell line against him in bitter combat. Hammer set up 40 cultures of lymphoblastoid cell lines from the liquid nitrogen bank. But when the mighty Hammer saw the cell lines arrayed there in the incubator, 'the heart was shaken within him; to avoid death he shrank back into the host of his companions. As a man who has come on a snake in the mountain valley suddenly steps back, and the shivers come over his body, and he draws back and away, cheeks seized with a green pallor; so in terror of the culturing warlike Hammer lost himself again in the host of his companions.' Then, Zeus sent him Dream, and Dream stood beside him as he slumbered and appeared to him in the likeness of his shimmering-gowned wife, speaking to him with winged words that he should return to his backyard solarium in Arizona lest the powerful cells might overwhelm him. And mighty Hammer obeyed the words of Zeus.

Then, as the icy Northeast storms raged over wide-wayed New York causing the chariots drawn by the swiftly running horses to spin on their wheels, the stalwart Ellis, 'like a lion who comes on a mighty carcass, in his hunger chancing upon the body of a horned stag or wild goat; who eats it eagerly, although against him are hastening the hounds in their speed,' casting caution to the wind and daring the perverse realms of the New York City subway system, set his brave heart against the tundra-like conditions, won the pinnacles of the cultures and lovingly cared for the abandoned cell lines. With the keen help of the Bulgarian princess glancing-eyed Proytcheva, stalwart Ellis and stintless Proytcheva produced 49 cell pellets. These pellets were sent to flowing-haired Hammer in Arizona far beyond the rim of the wide sea. Mighty Hammer swore a solemn oath that he would by the painful labors of his own two hands purify the hereditary material for all the mighty hosts who would have use of them.

Yet exhausted by these Gargantuan efforts, godlike Ellis also prepared blocks on over 38 cell lines for pulsed-field gel electrophoresis. The Herculean task is not over. First, frozen lymphocytes are available for establishing cell

lines, and eight such cultures are in the process of becoming established (they also will need to be expanded to make cell pellets); secondly, a number of already established cell lines need to be expanded for cell-pellet preparation, namely those that were not opened by the haughty Hammer, those that soon will arrive from the Coriell, and those that failed to expand in godlike Ellis' strong hands; thirdly, blocks must be prepared on 38 remaining cell lines. By completing these daunting tasks, we will arrive at the stated 76 DNA preparation objective. Warlike Hammer and humble and modest Ellis prepared complete hecatombs to Zeus that he might accomplish these things by June 1.

When the DNA preparations have been made, we will distribute the set of DNAs to faithful collaborators who have been waiting so patiently and long. Investigators will be assigned one or more Y chromosome loci to genotype in the entire collection. The results of the collective genotyping will be published in this Newsletter and will be available through the internet (server address to be announced). We sincerely hope that the arrival of DNA from the YCC will spur some individuals to new heights of altruism, causing them to submit more cell lines to the Repository and that others will join in battle against the deadly DNA polymorphisms that lurk with uncanny invisibility on the woeful Y chromosome.

Nathan Ellis

Quotations were copied with some modifications from Richard Latimore's translation of the *Iliad*, University of Chicago Press, 1951.

RESEARCH INTERESTS

RESEARCH INTERESTS is a feature of the YCC Newsletter wherein investigators report their research initiatives and activities (Reports) and publish results (Brief Communications). The editors welcome contributions to this section of the newsletter for publication in the next edition of the YCC Newsletter.

Report

Report from Lutz Roewer and collaborators

We are establishing Y chromosomal haplotypes using five different microsatellites: *DYS19*, *DYS385* and the loci defined by the probes YCA 1-3. We will use these five-locus Y haplotypes to describe male gene flow within and between populations:

- German populations (estimation of haplotype frequencies and mutation rates), application of Y fingerprinting for forensic stain analysis and paternity testing (e.g., deficiency cases).
- Establishing Y haplotypes for 2 isolated groups of Yanomami Indians from Southern Venezuela and Northern Brazil (approx. 100 DNA samples from each group extracted from hair) (with Gabriele Herzog-Schroder, Andechs, Germany).
- Examining "Austronesian" Y chromosomes (so far 115 HMW-DNA samples from Trobrianders of Papua New Guinea and 10 samples from the Ami of Taiwan) (with W Schefenhovel, Andechs and M. Hickson, Honolulu)

We will compare these results to those from the HLA class II region and mtDNA.

Lutz Roewer, Manfred Kayser, Marion Nagy, Institut of Forensic Medicine, Humbolt-University, Berlin.

Brief Communications

Three Y-specific polymorphisms in populations of different ethnic and geographic origin

The aims of our studies are a) to assess the genetic relationships between European populations and search for traces of past migrations and colonizations with particular regard to the diffusion of the Neolithic cultures and the Phoenician culture; b) to verify our previous hypotheses about the Asian Y chromosome haplotypes which colonized the Americas by the analysis of some Oriental populations; c) to search for micro-heterogeneity in Sardinia with respect to the different linguistic groups; d) to complement the

information we have already obtained through mtDNA analyses, by clarifying the anthropological position of particular groups such as the Tharus of Terai (at the border between Nepal and India) and by searching for Y-specific polymorphisms that distinguish different populations (e.g., in Hindus, where mtDNA distinguished Hindus from other Caucasoids).

We have analyzed *TaqI* RFLPs with the probes p12f2 (*DYS11*) and p49a,f (*DYS1*) in more than 2,500 individuals including 77 Turks from Istanbul and 131 from Konya, 88 Lebanese, 85 Tunisians, 58 Algerians, 215 Sardinians, 84 Sicilians, 235 Continental Southern Italians, 90 Greeks, 56 Albanians, 100 Hungarians, 90 Basques, 40 Dutch, 76 Hindus, 96 Tharus from Nepal, 40 Indonesians, 30 Malaysians, 193 Chinese, 69 Ethiopians, 64 Mandenka from Senegal and 86 Cameroonians. We are in the course of analyzing people from different areas of the Caucasus and from Southern Spain. We have also started to examine some of these samples at the *DYS19* locus (Y-27H39 STR).

Some results

- The p12f2 8kb fragment was not found in Orientals and in African Biaka; it still maintains its characteristic as a Caucasoid allele. It is most frequent in Near-Eastern people¹ and South-Eastern European populations^{2,3} and least frequent in North-Western Europeans (about 4% in the Basque⁴).
- As for 49a,f RFLPs, the frequencies of several haplotypes are characteristic many of the populations we have studied. For example, Ht12 is most frequent in Sardinians⁵; Ht7 in people of Asia Minor and the Near-East; and Ht5 in Algerians and Tunisians. Consequently, haplotype frequencies are useful for detecting and evaluating traces of migrations and admixture (e.g., there is a relatively high frequency of Ht5 in Sicilians). Of interest is Ht15 which was always encountered in complete association with the p12f2 10-kb fragment (280/280 observations) and only sporadically found in people of non-European origin⁶. It is virtually absent in Northern Africans⁵ and in Near-Eastern people and it shows a gradient of frequency increasing from South-Eastern to North-Western Europe with its maximum value

of more than 60% in the Basque⁴. This gradient has an opposite direction of that of the p12f2 8-kb fragment. Ht15 is therefore the best candidate to be a proto-European haplotype, while the p12f2 8 kb fragment could have spread with the Neolithics and subsequently with the Indo-Europeans (this fragment was found in Hindus) and the Phoenicians (high frequency in Tunisia and Algeria⁷).

- The analysis of the association of the p12f2 fragments with the 49a,f haplotypes has shown that 85% of the p12f2 8 kb fragments are found in association with Ht8, Ht7 and Ht24. Since 30/30 of Ht24, 124/130 of Ht7 and only half of Ht8 (83/168) were associated with 8-kb fragment, the most economical hypothesis (in which we only allow changes of a single band in the 49a,f hybridization pattern) is that all Ht24 chromosomes and almost all of Ht7 chromosomes can be derived from an Ht8 chromosome that carried the 12f2 8-kb fragment.

- We previously had suggested that p49a,f Ht13, Ht18 and Ht63 could be considered founding haplotypes in Native Americans⁸. This hypothesis finds strong support in the observation that Ht13 is the most frequent haplotype in Chinese (30%), followed by Ht18 (12%). Ht63 is found in Chinese; however, it is present at a lower frequency (3%).

A preliminary analysis of the *DYS19* locus has shown differences between populations. Five different alleles are known at *DYS19* (A-E), and these alleles appear as fragments of 186, 190, 194, 198 and 202 bp, respectively^{9,10}. As already observed for other Caucasian groups⁹⁻¹¹, the B allele is the frequent (51%) in our continental Italian sample (n=97) and reaches a very high frequency (80%) among Basque⁴ (n=73). Sardinians (n=91) are exceptional in that alleles B, C and E have similar frequencies, each accounting for about 25% of the sample. Chinese (n=97) are predominantly C (54%) and Senegalese (n=78) prevalently C (41%) and D (31%). An fragment of 178 bp was found in one Tunisian but accounts for about 20% of Ethiopians chromosomes (n=75). A previously unrecognized fragment of 208 bp was observed in one Algerian and in 2 Chinese. These studies are continuing on other populations; for example, on people from different areas of the Caucasus

and Southern Spain. We intend to integrate our analyses with other Y-specific polymorphisms.

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The relationships of Y chromosomes determined using five RFLPs and the YAP element

In a previous publication¹², eight polymorphic probes were analyzed on a panel of 91 Y chromosomes. The highly polymorphic probes allowed each Y chromosome to be distinguished, but were not very useful for establishing evolutionary relationships among the different haplotypes. Therefore, a subset of the loci were selected based on the criteria that the polymorphisms were binary and likely to have arisen a single time in human evolution. This reduced the data to four haplotypes consisting of the presence or absence of the 6.0 kb and 4.1 kb aliphoid units, and the 92R7 and M911 polymorphisms. Phylogenetic analysis resulted in a single tree with four branches leading to 1) group 2, group 4 and two pygmies, 2) the !Kung sample, 3) group 1 and group 3, and 4) two Amerindian samples. We have now included the polymorphisms at the DXYS5Y and YAP (DYS287) loci to this data set. Both of these polymorphisms have been generated by a single mutational event making them ideal for evolutionary studies^{13, 14}.

Numbers 1-12, 14-55, 57-79 and 93-100 (n=85) were tested by hybridization of a YAP probe to *TaqI* and/or *HindIII* digested DNA. 19 samples were also genotyped at the YAP locus by PCR (see following Brief Communication). The results obtained by PCR were without exception consistent with the results obtained by hybridization.

We found that the YAP element was present in 19/19 group 4 chromosomes, and it was absent from 66 chromosomes examined from other groups (Table 1). Although this correlation may not hold in a wider sample of individuals, for the present, group 4 is defined by the presence of the YAP element.

Table 1 The presence or absence of the YAP element at *DYS287* in 85 chromosomes from four groups¹².

Group	YAP ⁺	YAP ⁻
1	0	30
2	0	29
3	0	4
4	19	0
other	0	3

By including these polymorphisms, two more haplotypes have resulted: Group 4 haplotypes are distinguished from Group 2 and Pygmy haplotypes by the presence of the YAP element, and a single chromosome from Japan was distinguished from other group 2 haplotypes by the presence of the Y2 allele at the DXYS5 locus. A parsimony analysis resulted in a single tree with six steps and a consistency index of 1.0 (Figure 1).

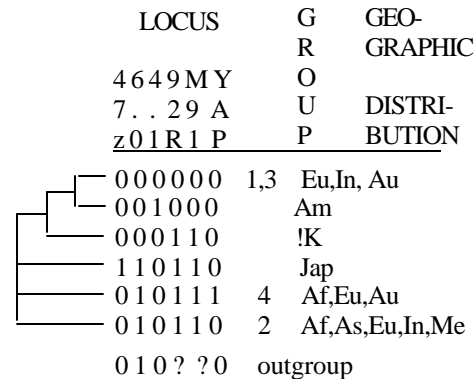


Figure 1. Parsimony analysis of six Y haplotypes. The six loci included in the haplotypes are indicated as 47z (DXYS5Y; 0=Y1, 1=Y2), 6.0, 4.1 (pY? 1; 0=absence and 1=presence of either 6.0 or 4.1 kb fragment), 92R7 (0=6.7 kb, 1=4.6 kb), M911 (0=33 kb, 1=55kb) and YAP (0=YAP⁻, 1=YAP⁺). The assumed ancestral states at four of the six sites are shown in the outgroup, which was used to root the tree. Chromosome groups are as defined in Mathias et al (1994). The geographic distribution of each haplotype is indicated as Eu (Europe), In (India, Sri Lanka), Am (America), !K (!Kung), Jap (Japan), Af (sub-Saharan Africa), Au (Australia), As (China, Japan, Malay, Cambodia), and Me (Melanesia).

Although the ancestral states for the 92R7 and M911 polymorphisms are unknown,

comparison of the ancestral states of the other polymorphisms with the extant groups shows that group 2 most closely resembles the presumed ancestor. This is consistent with the widespread geographical distribution of group 2 Y chromosomes.

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PCR-based detection of the Y Alu polymorphism (YAP)

The Y Alu polymorphism, or YAP (*DYS287*), is caused by the presence or absence of a ~300 bp Alu element at a specific site on the human Y chromosome¹³. Previously, this insertion polymorphism was detected by hybridization of *TaqI*- or *EcoRV*-digested genomic DNAs with a 500-bp probe (pYAP-0.5). To facilitate the detection of this polymorphism, oligonucleotide primers have been designed to unique sequences flanking the site of the YAP element insertion¹⁴. In this communication, I report the primer sequences and conditions for detection of the YAP element by PCR.

Primer Sequences:

5' CAGGGGAAGATAAAGAAATA- 3'
5' ACTGCTAAAAGGGGATGGAT- 3'

PCR Conditions: PCR was carried out in a total volume of 25 µl containing 100 ng of genomic DNA, 0.12 µM each primer, 0.2 mM each dNTP, 2.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.5 units Taq DNA polymerase (Cetus). The cycling conditions were 94° C for 2 min and 30 cycles of 94° C 1 min, 51° C 1 min, 72° C 1 min.

PCR product assay: The amplified products, either 455 bp (YAP⁺) or 150 bp (YAP⁻), were resolved on 2% agarose (fig. 1).

M YAP⁺ YAP⁻ female M

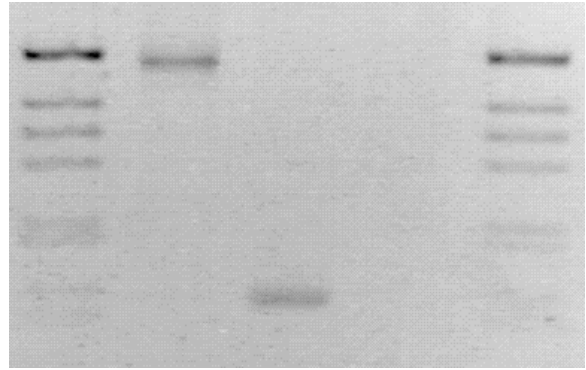


Figure 2. Agarose gel electrophoresis of PCR products from a male with the Alu element (YAP⁺), a male without the Alu element (YAP⁻), and a female. M, molecular size marker. [Run in 1X TBE.]

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POLYMORPHISM UPDATE

This section of the Newsletter draws the attention of readers to new Y polymorphisms and to additional information about known polymorphisms.

New Polymorphisms

YRRM2, originally described as a candidate gene for the azoospermia factor *AZF*, was reported to be undetectable by PCR amplification in 50 out of 50 normal Japanese and 7 out of 10 Americans (Nakahori *et al.* 1994; see Appendix 3 for references). Since this polymorphism is detected using PCR and the locus is either present or absent, it is potentially a very useful marker. Which Y haplotypes contain *YRRM2*? Does its gain/loss represent 1 or many events?

DXYS156Y is a microsatellite consisting of repeats of the pentanucleotide (TAAAA)_n and has homologous loci on the X and Y chromosomes (Chen *et al.*, 1994). In a survey of 36 Y chromosomes from the CEPH families, 1 allele of 160 bp was found and the other 35 alleles were 165 bp long.

Additional information about known polymorphisms

YAP, the Y *Alu* polymorphic element, has been used in several studies but now receives its most detailed description (Hammer 1994). The *DYS271* (detected by PCR using the

oligonucleotide pair sY81) point mutation described in the last Newsletter has now been published in *Human Molecular Genetics* (Seielstad *et al.* 1994), and an abstract summarizes work in progress on the minisatellite *MSY1* (Jobling *et al.* 1994).

Further information is available about the population distribution of *DXYS5Y* (detected by the DNA probe 47z) alleles (Lin *et al.* 1994), the microsatellite *DYS19* (Muller *et al.* 1994) and the *DXYS1Y* (detected by the DNA probe pDP31) duplication/triplication (Spurdle and Jenkins 1994).

Chris Tyler-Smith
