Marabou Discussion on Nutrition and Human Evolution

A referenced summary of the discussion with some appropriate references.

Compiled by Philip James on the basis of the recorded discussion

The size of human migration out of Africa

Philip James*:*

Mark Stoneking presented an astonishing array of data and material from many different sources. I had only a preliminary understanding of the topic of human migration based on a paper over 10 years ago, describing how our understanding of population migration was initially based on such elegant studies as those by Cavalli-Forzi describing the migratory shift in cultures (1). Then with early genetic mapping work the decline in the presence of primitive primate genes in humans seemed to be in proportion to the log of the distance of the communities across the world from Addis Ababa (2). The authors were able to calculate the very large size of the African populations and estimate each community's expansion in size and then delineate the small genetic sub-group who migrated out of Africa and on across the Middle East to Asia. Now we see different maps of population movements and I heard a claim that only 100+ people managed initially to move out of Africa. Mark Stoneking now also talks about a backward migration into Africa. Who wants to elaborate on this picture and perhaps consider the analyses for Neanderthal genes in homo sapiens?

Mark Thomas: I don't know why the authors chose Addis Ababa in that study – it doesn't seem to be a good proxy location for the source of human migrations out of Africa, but it was a plot of the decline in heterozygosity and diversity. The main underlying point in that paper was that it was continuous and that there weren't any sharp jumps so the population variation is pretty much spread continuously with only some lumpiness. The arguments about the 100 or so people migrating out of Africa is not strong – I don't know what Mark Stoneking thinks? I also wonder why people say "when humans left Africa" because most humans at the time had the good sense to stay behind anyway – only a few freaks actually left the place! With Neanderthals there are actually a number of Neanderthal genes transferred with clear evidence of multiple events leading to their admixture with homo sapiens.

Mark Stoneking: Just to clarify this issue about the number of individuals that left Africa. As population geneticists, whenever we talk about numbers of individuals e.g. leaving Africa, we are always implicitly referring to an 'effective' population size. So basically the amount of genetic diversity that you see in populations out of Africa can be explained by an ideal randomly mating population where everyone is unrelated to everyone else with somewhere between 100 and a 1000 individuals involved. But if you extrapolate from the difference between these ideal population genetic models and the numbers actually leaving Africa, it is probably of the order of at least 3 to 5 times greater in number terms but this is a rough guess.

Mark Thomas: The difference between effective and census population size is sometimes put at between 10 and a 1000 fold difference, so the numbers could have been a lot more. I agree with you - we should be aware that if the effective population size was 100 that could have still reflected the impact of 10,000 individuals migrating.

Mark Stoneking: Sure, it all depends on the social structure.

Mark Thomas: And the degree to which they are really isolated.

Sarah Tishkoff: We need to consider how many migrations of modern humans there were out of Africa. Was there a distinct Southern migration? When did it occur? What was the source population or populations in Africa? We know of the existence of Neanderthal and Denisovans but there were others - in fact there were a lot of different archaic populations. We have not discussed Homo floresiensis, the so-called 'Hobbit' (which was a bad name I am sure for him) but it had a funny brain and was about 3 ft. tall and it was on the island of Flores in Indonesia. There was a big debate as to whether they were modern humans.

Then people said no we just need to consider Homo erectus but at a recent meeting a paleoanthropologist claimed Homo floresiensis was not Homo erectus but more ancient and I think there was even more ancient "divergence" in the evolutionary tree.

Another thing people are starting to work on when considering changes in the admixture of ancient DNA over the last 10,000, 20,000 and 30,000 years is the evolution, for example, of lactose tolerance - we should be able to find the genetic variances associated with them. We could then model over time this evolutionary trend.

Dietary diversity and human evolution

Mark Thomas: I am not quite sure when it comes to modelling, which ancient DNA clearly anchors your information points with sufficient strength? We – and others - have developed methods for using ancient DNA to estimate the strength of selection for example. With ancient DNA you have much more power to detect signatures of natural selection than any of the methods we have discussed today using modern genetic data. You can then see the trajectories of genetic variance through time and estimate with much more power and precision the signatures of natural selection.

One of the basic limitations of using ancient DNA is the difficulty of getting good data but we are more or less doubling the number of ancient genomes every year, at least for Europe. The second limitation basically relates to the models. It has always been the case in population genetics – the models have limitations. We know that natural selection is going on for things like lactase - Sarah (Tishkoff) and others have published many papers on this as a marvellous example of natural selection in response to diet. But we still don't know to what extent it is just natural selection and how much it reflects demographic shifts in populations, because demography can confound patterns of natural selection. So, for example, if you have a genetic variant on the front of a migrating wave then that genetic variance can increase in frequency in the new habitat and leave a signature that looks just like natural selection when it is not.

Anne Molloy: That point is really important because a lot of us in nutrition are looking at polymorphisms and we are trying to say they were selected for or against because of something in our environment. I hadn't realised before that if something occurs in 2% of the African population but in 17% of Mexicans this does not mean that the difference reflects great selection process. I never knew why African populations were so heterogeneous - presumably the population spread explains a lot of the different polymorphic frequencies. It doesn't necessarily tell us why they are associated with disease – that is a different question.

Sarah Tishkoff: The big challenge is how do you distinguish demographic based change from selective pressures? Both processes can leave a very similar footprint. But we can make analyses about selective pressures for something like lactose tolerance because it has such a whopping signature. But let us consider the whole thrifty gene controversy and the problem of detecting polygenic selection. When there are many genes influencing a trait we don't necessarily expect to see a signature at any individual gene because they are not having a major effect. So people like Jonathan Pritchard and Graham Coop (3) and some other theoretical population geneticists have developed methods where you look for subtle shifts in the frequency of alleles for polygenic traits. That can be a sign of selection but that is also conditional on assuming that you know what the genetic variants are that are influencing those traits and if you don't it is a challenge.

Mark Thomas: Everything Sarah says is correct: it is all about power. If you have a powerful selection process it is easy to spot; if you have weak selection, it is more difficult but there are methods we can develop to discern the effect. So in many studies on lactase (4) and countless other studies for example by Ian Mathieson and colleagues (5) we can see just how strong selection is. The rest of the studies are mostly to do with skin pigmentation in Europeans (6). But just to put this in perspective, evolutionary biologists, such as ourselves, have estimated that selection for lactase is around 5 to 10% per generation which is massive!

Philip James: Slow down! Why is this so obvious to you?

Mark Thomas: Because we know roughly how big a difference that would make in terms of the rate of change of allele frequency through time (7). So we estimate 5% -10% as the rate over the last 7,000 years or perhaps more recently - we are not entirely sure. This analysis relates to Europe (8) but it is a similar selection spread in Africa (9). That is a faster spread than skin, hair and eye pigmentation genes. We conservatively assume 5% per generation based on data on causes of death in 2013 in Britain and the age at which they die. I decided to work out how much selection there would be on a gene - if such a gene could exist – that made us immune to all known cancers. And the selective advantage would only be about 0.13%. So that is 38 times lower than what we see with lactase persistence over at least the last 5,000 years. If we add a gene that makes us immune to suicide, then the change in allelic frequency is about 0.1%. Suicide is a big killer of young people. With immunity to cardiovascular disease and stroke: the selective advantage would only be 0.057 i.e. 88 times less than lactase persistence. And if we had a gene that made us immune to death, so it made us immortal, then the selective advantage on it today would only be about 1.2%. So that is still 4.2 times less than the huge selective advantage of lactase. And I am making these calculations conservatively, so the selection strength would actually be less with these other factors..

Sarah Tishkoff: Has that got something to do with reproduction?

Mark Thomas: Yes -the reason is exactly as Sarah said: it is because we do die, but we don't generally die before we have reproduced.

Philip James: Hang on a minute! Before you were born, Mark, I worked in Jamaica trying to stop malnourished children dying at an enormous rate. One of their main problems was diarrhoea induced in part at least by malnutrition induced lactase deficiency and malabsorption (10) as well as gastroenteritis (11). I showed these children can recover with suitable rehabilitation. At that stage in Africa, Asia and the Caribbean, anything up to 50% of children below the age of 5 were dying. So I mean if you have a 50% death rate before reproduction then factors influencing these deaths will have enormous impacts. .

Mark Thomas: OK – my analyses were based on UK births and deaths in 2013. I also did some calculations for populations – these weren't in Africa, they were in India from, I think, the '60s – and the capacity for selection was much higher in those populations because more kids were dying. The proportion of kids that die is the capacity for selection based on survival. But again, this is just to put those values of 5% or 10% into perspective. They are enormous - absolutely enormous - so if ever you wanted to ask the question are humans still evolving, well clearly they are. If you want to ask what matters – diet is the big one that matters: it just keeps on coming out again and again and again.

John Speakman: So how do those selection figures look if you factor in impacts of reproduction? Can you actually do that?

Mark Thomas: Absolutely: there are other ways – there are three levels at which you can get genomic selection. One is survival; one is fecundity and the third is the gamete level sperm competition. So you could have somebody that gets a big selective advantage just because they are good at mating. But my 5% -10% figures are based on survival, not on mating.

John Speakman: So total selection on any gene is not just a question of survival potential?

Mark Thomas: True! I simply want to illustrate how massive a 5% selective advantage is and the estimates are based on very different methods e.g. haplotype decay or based upon ancient DNA or based upon more spatially explicit modelling – it doesn't really matter what we are doing: we are basically coming out around those figures. It is really just to nail home how strong it is.

Patrick Stover: You mention the word diet: how do you know that that polymorphism wasn't driven by having a clean source of drinking water – and that it was totally unrelated to diet?

Mark Thomas: A clean source of drinking water is an aspect of diet and I completely accept that one of the plausible explanations for why lactase was so advantageous was because if you don't mind getting brucellosis, milk is actually a good source of clean fluid. We don't know which are the specific selective factors - it is almost certainly a constellation of selective advantages. They were almost certainly multiple and different in Africa from those affecting Northern European populations. Almost certainly clean fluid is much more important in, for example, Arabia where lactase persistence is at high frequencies and possibly in arid African regions. Probably not so important in Northern Europe – perhaps calcium and vitamin D are more important in Northern Europe. We don't know: there are many hypotheses for why milk drinking with lactase persistence is advantageous.

William Leonard. The general public when considering the evolution of nutrition think about paleo-diets. And yet the remarkable fact is that you have a broad diversity of recent nutritional adaptations which have, I think, enormous potential for what we are going to be discussing later – those larger health implications of the mismatches that we are seeing between modern society and our evolutionary past.

Amanda Henry: A lot of the theories that we have been proposing for changes in human evolutionary time span and the diet of early hominins are probably completely wrong!

Philip James: How do you know they are wrong?

Amanda Henry: Because even among the paleoanthropologists there is no consensus. People will look at the same set of data and come up with widely different conclusions in terms of what macronutrients we have gotten from the environment, what proportions these macronutrients were in and the importance of these macronutrients in human evolution. For example, I was a bit concerned about the idea that meat was the important nutrient 2 to 3 million years ago. We have no idea how much meat was eaten and although consumption rose it was not the fundamental food source for any hominins until very recently. So we need to be careful when making these interpretations about shifts in diet in relation to human evolution because there was not one single pattern. Furthermore, there was no single paleo diet ever but a range of environments that humans lived in and had diets that were adapted generally to those environments. I think the human state is more about flexibility and an ability to acclimatise to a variety of habitats and diets than it is about evolution in response to a specific diet.

Philip James: That is very interesting because I remember Michael Crawford claiming that the spread of the human species was dependent on their need for enough n-3 fatty acids to build their brains and therefore they could only migrate around the seaboards into the Middle East (12,13).

Mark Thomas: Amanda Henry's point is very important – we have discussed various aspects of adaptation, physiology and diet, but nobody has really made any statements about what they think our diets were, at particular points in time. Clearly human evolution depends on when and where you set the clock running, but if you want to make it interesting, set the clock running at the time of the invention of stone tools. That seems perfectly reasonable but then there have been serious changes over that period. And fundamentally we don't know what a Palaeolithic diet was. The only thing we do know – as you have just pointed out and Sarah pointed out – is that there was no one Palaeolithic diet. That is the only thing that we can be reasonably confident about. I have dug into the literature on this and there is a lot of speculation about there having been more potassium, less sodium, more protein, less this, more that but I haven't seen a single publication that has systematically tried to quantitatively estimate either nutrients or the evolution of foods over time.

John Speakman: We cannot even estimate accurately what people eat now so how will we ever know what somebody a hundred thousand years ago was eating?

Philip James: I thought that examining fossils and finding odd nuts and food remnants in fossil sites was the basis for all the claims?

John Speakman: I think from isotopes you can probably tell a broad stretch of, you know, whether there is more C3 or more C4 in the diet.

Philip James: A number of people are mumbling "rubbish" but keep going John!

John Speakman: I agree there is nothing quantitative but just a crude idea of previous diets.

Andrew Clark: There was the recent paper in Nature on the calculus of the teeth in the Neanderthal showing that the peoples living in the El Sidrón Cave were mostly vegetarian (14).

Amanda Henry: I work with dental calculus – it doesn't show the entirety of diet at all, not even close. So Neanderthals in El Sidrón probably did consume a lot of plants, but that was certainly not everything that they ate. None of the ways for assessing diet in the archeological record are robust and complete. Yes, C3, C4 analysis is great for telling broad food categories, but whether that was plants or the animals that were eating those plants is very difficult. It is not beyond the realm of possibilities that hominins were actually eating grass. Some of the grasses are actually very nutritionally advanced and I think that is another issue that we haven't grappled with – we don't understand a lot about the nutritional quality of wild foods; most people here have been working with domesticated foods which are markedly different from what was available in the landscape. So domesticates do not represent what would have been available in pre-agriculture times.

Philip James: It is worse than that because over the last 100 years we human beings have dramatically altered the diversity of human diets. For example in the United States there used to be over 200 varieties of cabbage while now there are only about 4 mass produced varieties and we all know that the human race now seems to depend on 10 crops – mostly cereal crops. We also know very little about the diversity of plant composition becuase most of the estimated analyses have been extrapolated from either British or American Food Composition Tables mainly derived from the 1940s to '60s

Richard Johnson: I don't think we should be too negative, but use not just the archaeological record but also our undrstanding of the molecular evolution record, of comparative physiology and so forth.

Mark Thomas: You are right – we shouldn't throw the baby out with the bathwater! Obviously you will be aware that a lot of people have talked about paleo diets. So we've reviewed the evidence and actually looked at hunter gatherer diets, or records of what they had been eating throughout the year (15,16). These include groups varying from the Circum-Arctic to the equatorial rain forest. We also took modern diets – from all round the world, from Hong Kong, South Africa, Brazil and so on. Also there is one archaeological site where there does seem to be good dietary information which is at Ohalo_II in Israel – an upper Paleolithic site that has amazing preservation of plant, fish and animal remains (17). We tried – using databases on nutrient contents of different foods –to work out very approximately for each calorie-normalised diet the proportion of different macro and major micronutrients in these diets. We did a very simple, multi-variant and principal components analysis. At least if hunter gatherers are a good proxy for ancestral diets, then they were really, really diverse; and you shouldn't be surprised because from the Circum-Arctic to the Equatorial rain forest you would expect real dietary diversity. Perhaps the most interesting thing is just how compact in terms of nutrient ranges modern diets are. So what is pulling modern diets away from the kind of diet consued by hunter gatherers? They contain higher sodium, sugars, somewhat more complex carbohydrates and things like theobromine found in chocolate, The exploratory data gives us some idea about the diversity of ancient diets.

Jere Haas: We seem to have evolved as a very adaptable species. Just looking at the range of nutrients in the hunter and gatherers' diets we realise that each diet failed to create a separate sub-species or race. I think we as a species could adapt in the short or long term to the dietary and environmental changes and much of those adaptations are probably social, cultural and behavioural; as well as relating to biological plasticity. In severe cases there may have been natural selection.

Bill Leonard: We have done similar analyses i.e. comparing macro-nutrient distributions of diets of subsistence level populations to the current recommended macronutrient distribution. You then find the diets of many hunting and gathering and subsistence level populations are very much at the margins of what the current recommendations are for healthy lifestyles (18).

Mark Thomas: We found that almost 45% of the variation in the distribution of 32 nutrients is explained in just two dimensions which means that the nutrients in different diets are highly correlated. This aggregation of nutrients in different foods is important.

Carel van Schaik: I could make the same point about the diet of my great apes – there is no great ape diet and what we should be looking for is the rule of plasticity that you follow. So we should be thinking about, for instance, protein prioritisation: how long has that been around and how strict was it – and stuff like that. For instance, the archaeology shows that Pleistocene hunter-gatherers knew obesity quite well. Think of the Venus figurines – I think they started some 30 thousand years ago. These guys knew what fat people looked like. So it suggests that things didn't change so much in practice. My guess is that we have been protein prioritisers for a long time and that when we had a chance to eat more, we piled it on.

The validity of analyses of single archaic individuals

David Rosenblatt: There was more than one Neanderthal population and I am concerned that we make so much from analysing just a few ancient skeletons. Yet we know there is a large variation in the human population, so how much information can we infer about a population from the sampling of a simple individual? Maybe Mark Stoneking can explain again how one can extrapolate from measures on one person to the population.

Mark Stoneking: There is a sort of inherent assumption that when you get a single fossil and its genome sequence it is somehow representative of the entire population or species from which the sequence comes. You actually need additional sequences in order to tell whether it is representative or not, But nonetheless there is still a very rich history that we are just beginning to learn how to work out that we can derive from a single genome sequence. It turns out that every individual's genome sequence contains a complete history of the population size change over time as displayed in that sequence. I confess I don't understand it fully but the basic idea is that you can divide the genome into homogenous chunks. You go along the genome and along the chromosomes until you reach a point where you can say, OK, I can no longer explain the history of this chunk by a single history – there must be a separate history and that's where the combination event has occurred in the past. So you go through and divide the genome up into chunks of different sizes. Then you take and compare the chunks of the same size and ask how much diversity is there in those chunks? The length of the chunk is proportional to its age – long chunks are recent, short chunks are old. The amount of diversity within a chunk is proportional to the population size at that time. If it was a big population size, there will be a lot of diversity in those chunks; a small population has less diversity in those chunks. Then you can mathematically estimate the diversity and translate the findings into what we population geneticists call 'effective population size' which may or may not tell you anything about the actual population size – but it reflects the amount of diversity. You can plot this over time and in your genome sequence there will be a record going back 3 to 4 million years and the population size change over that time. It really is remarkable! You can do it from a European, an Asian or an African perspective and you can find that prior to about 50-60 thousand years ago, you get exactly the same history. You do the different analyses for the different continental populations completely independently and you get exactly the same results. Only around 50-60 thousand years ago did sequences suddenly change between individuals and then subsequently you will see differences in the genetic histories. The Neanderthal gene sequences looks exactly like the modern human until about 500,000 years ago, and then it starts to diverge. So there is a rich history, even within a single genome sequence that we can make something out of.

Mark Thomas: Can I try to explain in a different way? What Mark [Stoneking] is talking about is a method called PSMC (pairwise sequentially Markovian coalescent) analysis which was developed by Richard Durbin (19) from the Sanger Institute in Cambridge. It is actually not as wonderful a black box as it is claimed to be because while specifying that it detects changes in population size it could simply be detecting changes in population structure under exactly the same population size – that has been shown recently very convincingly. I think a better way to see it is that an individual's genome provides you with a sample of their ancestors and these ancestor numbers double in each generation. Now funnily enough it is not a sample of all the ancestors, because they have ancestors from whom they have inherited no DNA even from a few generations ago. But when you think about our species, everybody in the world shares a common ancestor with everybody else in the world only around 4-5 thousand years ago. This time is extremely recent – so what that means is that your genome, individually, is an extremely good representative of the whole human species today. So actually, these single ancient genomes, even from, for example, a little bone, gives the most amazing coverage – I mean one of the best coverage of any of these ancient hominids remains. So it is not just reflecting an individual but provides a sample of ancestors that themselves joined up with each other at incredibly rapid rates, going back through time. It is actually really representative of almost whatever it was in the population species and reflects the impact of large numbers of individuals.

Greg Wray: If you want to infer effective population sizes over time, you are absolutely right. But there are lots of other questions that are equally interesting such as the diversity across space and time, and it's really not so good for that.

Mark Thomas: Well it is not terrific either for population size.

Greg Wray: But a lot of the interest that many of us have involves things like local adaptation and then a single analysis is just a point estimate and is not going to give us much on that topic.

Diet again

Brian Fowler: The evolution analysis from genome change is very impressive but I am trying to get my head around the dietary aspect side – can we think of, not the detailed nutrition, but maybe generally features like omnivore, carnivore, herbivore? Can we do that? Then my question is, is there any evidence in the past - vitamin B_{12} is my interest – that subjects were enjoying or subjected to a vegan diet?

Amanda Henry: I think that if you use what we know about living apes today, and about current human nutritional requirements and the archaeological record we conclude that humans have always been omnivores. Even chimpanzees and great apes, are omnivores – they eat some component of meat and if not meat from vertebrates, then they are eating insects. So they are getting proteins from that source. So I very much doubt veganism was ever an option.

Bill Leonard: But I think there was an important change as hominids spread out onto the Savannah. Then animal material became a larger portion of the diet than we see in the great apes. Meat and animal material has an awful lot of concordant nutrients that I think were critically important to that stage of human evolution.

Amanda Henry: We tend to think of meat as involving only hunting antelopes, but insect and insect protein could have been extremely important and it would have been available in a lot of environments including in abundant quantities for forest-living primates and would have provided very similar – as far as I understand it – nutritional components to those of animal meat. So perhaps it was just a shift in the source of animal protein. Somebody is shaking their heads in the audience!

Stephen Simpson: As an etymologist I agree! In fact with Jessica Rothman and David Raubenheimer we published exactly that with our geometric analyses of hunter-gatherer diets (20). The contribution of insect protein and lipids to the human diet has probably

been underestimated appreciably. But another point: I want to come back to Carel (van Schaik) who showed in hunter gatherer diets his analysis of Eigan vectors and there is one thing that unifies different diets, including more modern diets, and that is that an increase in sugar and therefore calories is balanced by a dilution of protein..

Mark Thomas: Another interesting thing that I didn't mention is the concordant increase in both the fat and carbohydrates as one assesses differences between ancient and modern diets. Now if you analyse individual foods, the macronutrients go in the opposite direction. The correlation is almost unknown in nature.

Stephen Simpson: Except for breast milk. If you look at the level of nutrients, there are essentially an infinite number of ways of eating different, or mixing different foods to obtain a certain combination of nutrient intakes. But at the level of nutrition, if you also take into account the autocorrelations that are occurring inevitably between many of the nutrients, it probably distils down to a very small number of critical axes. Protein and non-protein energy, whether from fats or carbohydrate are the axes in the human diet. The percent total protein to total energy is remarkably consistent across populations so any small dilution of one by the other can lead to radical differences in energy intakes. There is no doubt that essential fats, essential carbohydrates, essential aminoacids are really fundamental – there is no question about that. But blaming, for example, the dilution of protein in the diet or the increased caloric intake from carbs or fats - one or the other - is axiomatically nonsense because we are dealing with the combination of nutrients in mixtures.

Richard Johnson: In addition to sugar and fat, I think you mentioned that sodium falls. Our group has an upcoming paper where we show that high salt diets can actually by themselves lead to significant weight gain and diabetes (21). It works through a serum osmolality pathway. There are now lots of papers that are coming out linking high salt diets not just with hypertension, but with the increased risk for obesity and diabetes. So it is something to think about that changing salt content may actually be playing a role in this evolutionary change that you are seeing here..

Mark Thomas: If you take the foods but not the diets, and then you look into the correlation matrix relating to sodium, and you ignore mineral sources of salt – so just food sources– then it correlates with many other major mineral groups. So the argument here is that you need lots of other minerals but you don't need to evolve an avidity for other minerals because sodium has a high correlation structure with those other minerals. So you just need an avidity for sodium. It is the same for L-glutamate – so we have a high avidity for L-glutamate, but you know you are going to get loads of other things with L-glutamate. Also we have a high avidity for sugar but we don't need sugar and sugar in an ancestral setting is unlikely to have been a major calorie source other than from honey, because wild fruits have very low amounts of sugar - it is only modern varieties that have high amounts of sugar. So in evolutionary terms fruit was not a good calorie source. Sugar was an amazingly good proxy for ascorbic acid (Vitamin C) which we clearly need. So you simply need to evolve an avidity for nutrients that co-occur with the nutrients you require, and that would work in an evolutionary setting.

Anne Molloy: Some sources of protein provide unbalanced aminoacids and others such as soya do provide enough of the essential aminoacid methionine – so how does this affect the supposed prioritization of protein in evolutionary terms?

Stephen Simpson: Protein is a 20 dimensional problem and for that very reason, trying to independently regulate 20 dimensions is an impossible regulatory task. So what you have got to do is to have some ceiling and floor mechanism - probably uncharged tRNAs - which allows you to know when you have got way too little or way too much over an extended period. Then we have developed a regulatory system which measures some proxy which will, presumably, involve one or more aminoacids. Our data most recently in mice intake suggest that the branch chain aminoacids are not among that signalling group. There is likely to be some lean associated signal, the protein in lean tissues, equivalent to that of leptin from adipose tissue. The only candidate molecule we have at the moment is fibroblast growth factor 21(FGF21) which is a low protein produced hormonal signal

(22,23). So integration of those signals is somehow measuring and detecting the quality of protein. Coming back to salt it is notable that sodium and calcium are the two obvious requirements which lock into a nutrient-specific appetite for micronutrients. Most other micronutrients are correlated with the salt content of natural foods so these other miicronutirients do not have their own separate appetite systems. Sodium and calcium tend to be regulated orthogonally to the rest of the dietary components because you couldn't afford, for example, with a shortfall in your dietary sodium, to increase your calorie consumption markedly to gain that little bit of crucial extra sodium. So it is much simpler to make a regulatory response by seeking out salt as a mineral at source, e.g. salt licks. But if you are in an environment where salt comes mixed at higher than optimal proportions with the rest of your macronutrients – and that is notably the case in the modern food supply, particularly with processed foods – you are going to have to eat more of it than you need to gain the things that you are regulating tightly, and that includes protein and macronutrients, and then you pay the price – that is where the consequence goes for health.

Patrick Stover: Just a quick comment on Brian (Fowler's) question about B_{12}. Animal based protein is a rich source of B_{12}. Some insect protein is a good source like termites; others, like houseflies, have virtually no B_{12}. So the amount of B_{12} is highly variable in insects.

Mark Thomas: Insects also contain carbohydrates as well as proteins. Humans have got four gut disaccharidases, one of which is trehalase. Now trehalose is a really weird glucose disaccharide that occurs primarily either as the blood sugar in insects or in fungi, and the vast majority of the people in the world maintain an active trehalase. Now the first rule of evolution is if you don't use it, you lose it. We haven't lost it therefore we must have been using it. So unless we have been eating a lot of mushrooms – we are eating some mushrooms, but you know there is more trehalose in insects. We must have had decent amounts of insects in our diet – it is a good signature of it.

Brian Fowler: Do we need to make the protein story even more complicated by adding small peptides in there or not?

Stephen Simpson: Possibly, but I doubt regulatory physiology is going to add all those things – whether there are protein peptides that serve a signalling function in protein appetite we don't know.

Hannelore Daniel. I have been studying peptide transporters in mammals for 30 years now. The peptides are not essential in terms of appearance in plasma or for any particular regulatory role. But in the intestine – as Stephen (Simpson) alluded to – we measure what is happening in the digestive phase and there the peptides play a crucial role. Peptides provide a surrogate for the quantity and quality of the protein because the amino acids are released immediately before uptake into the intestinal lumen itself. So the intestinal receptors are exposed and washed with all kinds of peptides and, of course, there are so many different types that play a crucial role for sensing processes, hormone secretion from endocrine cells (24) and so on but not at a systemic level.

Gema Fruhbeck: Has taste-receptor detection changed in evolutionary terms over time? You mentioned that taste receptors are well-expressed all over the digestive systems (25) and there have been anatomical changes in the length of the digestive system. Is it something of an advantage if you are able to detect different tastes and does this system have an evolutionary effect?

Sarah Tishkoff: Was your question how long have these mutations been around in evolutionary history?

Gema Fruhbeck: Yes; also if it has had any effect in driving evolution. So can we see the impact of an abiity to taste in a more precise way different aminoacids and different combinations of branch chains? We know animals have this attribute (26).

Sarah Tishkoff: We have been doing some analyses of this (27). We did whole genome sequencing of some hunter gatherer populations. The relevant genes tend to exist in

clusters of genes and they are very often targets of selection in certain populations that we have been studying. So I think that is an excellent outstanding question – the targets of selection are evolving and how much does that co-evolve with our changes in diet for example? Does one lead to the other? We also see a lot of changes to the olfactory genes so presumably this may also affect the types of food eaten – I don't know.

Stephen Simpson: Taste receptors are not only in the mouth but much of the entire gastrointestinal tract is filled with chemoreceptors that are detecting food as it travels downwards through the intestine (28). And those taste receptors are presumably part of a system that has to predict and estimate the nutritional quality of what you are just about to have, or have put in your mouth. Added to that, there are things like digestive enzymes, alpha amylase – 1(amy-1) in saliva and there is copy number variation in this enzyme. And the detection of sweetness in the mouth is a predictor of what the carbohydrate value of that food is going to be once ingested – and we are super-sensitive to very small quantities of sweet foods. If you put it in higher concentrations in the form of honey or very sweet substances, you are stimulating that system super-normally.

William Mair: This is not my area of expertise, but regarding taste chemo – receptors cells, a colleague of mine had a paper last year looking at the gut taste receptors' role in responding to parasites (29) and changes in the microbiome and infection in the gut. Tuft cells with their chemoreceptive receptors comprise a minor fraction of small intestinal epithelial cells and are putative quiescent stem cells. A huge expansion in these taste receptors in these particular cells occurs which then stimulates the immune response. So that would also change adaptation.

Sarah Tishkoff: Taste receptors in the nasal cavity relate to mucosal immunity in certain genotypes (30) and a colleague showed remarkable results where he looked at the phenylthiocarbamide (PTC) responsive genotypes and these correlated with their susceptibility to sinusitis - people who were prone to having very serious sinus infections had a specific genome type at the PTC responsive site involving the TAS2R38 gene (31). That was remarkable because it also raises the possibility that if these taste receptors are playing a role in, say, infectious disease resistance, their role may have nothing to do with diet but immunity from infections; this is where evolutionary pressures could also be important.

Animal Models

Leif Andersson: I have been working primarily on domestic animals and one of the studies we have been doing is looking at the wild boar and domestic pigs. We crossed them generating F1s and then F2s. This parallels Charles Darwin's similar studies on the wild boar and domestic pig in England around 1850 or 1860 (32). At that time these large white pigs were very fat because people bred them as an energy-rich food. But then after the Second World War there was an exceptionally strong selection process to produce leaner carcases so every year the back-fat thickness of pigs has got leaner and leaner in a linear way.

When we crossed our two breeds we noted that the F2s on average become fatter than the pure bred domestic pig. So what you would say is that genes from the wild boar were thrifty and were consistent with accumulating fat. We were then able to identify a couple of gene loci which explained the variation in body composition and the proportion of muscle versus fat. The major locus for that turned out to be the IGF2 locus. This is the major locus both for muscle growth and for the reduction in subcutaneous fat. This one locus explains about 20% of the phenotypic variance in this trait. Then we sequenced this gene region and compared the genetic profiles of the European domestic pig and wild boar. There are at least two sub-species of wild boar - the Asian and European. They split genetically about 1 million years ago. What proved fascinating was that the fatter European domestic pigs have the European wild boar allele haplotype whereas the European domestic pig with high muscle growth has the Asian haplotype. What you also need to know is that during the 1700s and 1800s the Asian genotype entered the domestic European gene pool because Europeans brought back Asian pigs to breed with the European stock. So our

European stocks are to some extent hybrids between the Asian boar's gene and the European domestic gene.

Comparing the haplotype groups from the Asian and European boars showed that there is a 1% sequence difference –about 30 KB of the IGF2 locus (33). There is a 300-nucleotide difference between the different boar strains whereas between the two types of fat or lean domesticated pig there is only a single base change in this 30 kb region. We then found that in the pigs that have been selected for high muscle growth they have an adenine substitution for a cytosine (34) and maybe 95% - 99% of all pork produced in the Western world comes from pigs that carry this adenine base. This gene structure is completely conserved in cattle, the horse, dog rabbit, human, mouse and rat (35) and this base change knocks out the interaction with the transcription factor that regulates the expression of the regulator for IGF2. So that when you mutate that base, the muscle IGF2 trebles in concentration and they grew more muscle. The mutation disrupts the interaction with a repressor and leads to the threefold increased IGF2 expression in postnatal muscle. And now we have mice into which we have introduced exactly this mutation and these mice get more muscle, so this proves that this is the causal mutation.

Philip James: Can you take that into a human context?

Leif Andersson: I think the problem with the data on Neanderthals and Denisovans is that we don't know exactly what their phenotype was and their potential contribution of genes to adaptive features in humans. We don't know if they were adapted to high altitude because basically we don't have their phenotype.

Sarah Tishkoff: I would be cautious about using domestic animals as a model system for what is going on in humans because it is often a much simpler genetic architecture than what is happening with these traits in humans. So we may learn some loci that are relevant but I know for height for example, in Europeans, it turns out to be so much more complex than what they were seeing in terms of the differences in dogs. In Europeans they found out that the growth hormone IGF1 pathway genes weren't playing such a big role in determining normal variation. So coming up with a candidate gene sometimes is useful but it may depend on the trait and the genes.

Leif Andersson: I don't think the number of genes controlling growth in dogs is strikingly different from humans. The difference is that you have very strong selection pressure in animals so when you start to select for lean growth in pigs, this is the single base change that has the largest effect. But there is much greater genetic diversity to explore in domestic animals than just looking at isolated features.

Greg Wray: We haven't really talked much about pleiotropy. I think human beings and pigs are extraordinarily complex organisms and we have heard repeatedly just how quickly we get into complexity. I think in a domesticated environment all sorts of things including low birth weight can be tolerated but this would not apply in the wild. It is true that in some traits like height in Northern Europeans, the effect sizes of a particular trait are tiny, but actually in some other populations we are seeing alleles with a bigger effect size. So, again extrapolating from one or two of these cases I think is dangerous.

Leif Andersson: In domestic animals there is selection against pleiotropy. One reason why this mutation is so variable in its effects is that it doesn't change, for instance, foetal growth. So at birth they are exactly like normal pigs, whereas some other mutations like myostatin knockouts have a big impact on calf survivals. This gene is paternally expressed but is not being affected by imprinting. When we knock it out in mice, we get about a 30-fold up-regulation in IGF2 expression, But what I think is fascinating is that the 16 base pairs in this mutated region is conserved in every placental mammal that we have looked at. Apparently there is a strong natural selection pressure that maintains the interaction between this specific transcription factor and that site.

Uricase and thrifty genes.

John Speakman: A semantic point - I think we should be quite careful about calling genes that affect fat storage 'thrifty genes' because the definition of a thrifty gene is a gene that is

being selected to allow that animal to survive periods of famine. As I understand it, Richard Johnson's gene has not been shown to influence the animal's ability to survive a period of starvation and it doesn't exist because of that selective event in the past. So it is a gene that may cause or contribute to obesity but I don't think you should call it a thrifty gene.

Ingemar Emberg: I don't see that this thrifty behaviour is in conflict with John's arguments that you can have other genes that are more directly involved in causing obesity. I think this locus is also subject to parental imprinting – which also has effects on body mass where there is the father's or the mother's allele that is dominant – does this relate to that phenomenon?

Richard Johnson: There are some assumptions John is making about thrifty genes. So his first assumption is that you have this gene that makes you obese but it really is a gene that protects you from famine and starvation and from loss of fat. The absence of uricase has been linked to fatness; humans do not have uricase and lost it about 15 million years ago (36). But the Yanomami Indian is not fat and primates that have also lost the uricase are not fat - they just augment the process to put on fat in response to a particular food.

Vilmundur Gudnason: The absence of uricase explains the higher uric acid levels in humans and its link with gout. But we have found 18 loci that also affect the concentration of uric acid in the human population (37). Obesity, cardiovascular disease and hypertension - in our studies on hundreds of thousands of individuals we do not find any relationships between genes that influence the uric acid level and their relationship to the level of blood pressure, blood glucose, estimated glomerular filtration rate, chronic kidney disease, or cardiovascular disease (38). There are also data from the Copenhagen Heart Study with 100,000 people suggesting that the relationship between uric acid and obesity or BMI is not causal. So I think that this is an extremely important thing when considering the uricase mutation and the effect of uric acid on these traits (39).

Richard Johnson: Most Mendelian randomisation studies that look at genetic polymorphisms controlling uric acid show that if you have a polymorphism that increases your risk for elevations in uric acid, then it predicts gout, but we can't show that the polymorphism predicts diabetes and that gets translated into the assumption that uric acid is unlikely to be a causal risk factor for diabetes.

All the biological studies looking at how uric acid works show that it is the intracellular not extracellular uric acid that drives the process. All the polymorphisms that have been linked with uric acid are basically transporters that actually have the effect of dissociating the relationship of serum and intracellular uric acid. So when you raise serum uric acid then the intracellular tends to be lower so you actually would not be expected to show an effect. So the way uric acid works is that it has to be intracellular to work and if you have polymorphisms that are not associated with increases in intracellular uric acid then you are not going to show the effect.

Vidar Gundersen: The deleterious effect of intracellular uric acid sounds very reasonable – the plasma levels then are not really relevant except for gout.

Richard Johnson: Kalle Moley's work (40) shows that the gene SLC2A9 encodes an intestinal uric acid transporter Glut9. This transfers hepatic derived uric acid from hepatic glycolysis and purine metabolism into the bile and accounts for 30-40% of uric acid excretion (the dominant route being the kidney). So when the transporter is not encoded blood uric acid levels rise and the metabolic syndrome with hypertension, hypercholesterolaemia and increased body fat results. So they concluded that the intestinal transporter is an important contributor to controlling blood uric acid levels. In the kidney the transporter controls the reabsorption of uric acid in the renal tubule but this is also controlled by a number of genes (41). SLC2A9 works by differentially knocking uric acid levels down in different organs with one organ, the kidney, driving uric acid reabsorption and the gut driving secretion.

Vidar Gundersen: I am not sure that that is explaining the uricase story!

Mark Thomas: The absence of uricase is a permanent feature in humans. So therefore it doesn't explain any of the variation in getting fat, but you are arguing that part of the mechanism of getting fat is by having a dietary regime that we are unlikely to have experienced in our evolutionary past?

Richard Johnson: Yes - so it is a genetic / environmental interaction.

Mark Thomas: So under a dietary regime of high fructose that we are unlikely to have experienced in our evolutionary past?

Richard Johnson: Correct!

John Speakman: The uricase story, however, has got nothing to do with the genetic variation that causes obesity because it's absence is a fixed feature. 35% of the variation between individuals' body fatness is just environmentally driven. Richard argues that we are all knockouts for uricase but that leads us to problems handling fructose and the amount of fructose then leads to obesity – all that is an environmental component and it has got nothing to do with the 65% of the variation between individuals that is the genetically based variation in the propensity to obesity.

Brian Fowler: Is not protein a confounding factor in the uricase story? One of the major determinants of uric acid production is protein intake. There are also rare genetic disorders leading to very high uric acid levels so can we learn something in the uricase story from those patients?

Richard Johnson: It is the purines within a protein intake with which we need to be concerned. The type of purine varies among proteins. Shellfish and other high RNA containing foods – shrimp for example –are the highest purine foods but the highest is actually beer! Now to come to the genetic disorders with high uric acid levels: the biggest one is called Lesch–Nyhan syndrome (LNS), associated with mental retardation and a lot of CNS issues which are confusing. The classical clinical phenotype of Lesch-Nyhan disease is defined by uric acid overproduction, motor dysfunction, intellectual disability, and behavioural problems including recurrent self-injury. But there are other clinical variants in which some of these clinical features occur but they have normal uric acid levels.

Mark Thomas: The thrifty gene argument is that it is an adaptation to changes in food supply. We are arguing that there is a biochemical mechanism that generates an adaptation to changes in a food supply that we haven't experienced in our evolutionary past. So why is that a thrifty gene? I just have this problem with invoking the concept of a thrifty gene when a mechanism is fixed, so cannot contribute at all to variation in fatness in modern populations, but responds only through a biochemical mechanism to a nutrient that is unlikely to have been significant in our evolutionary past.

Greg Wray: Neil used the term 'the thrifty genotype hypothesis' and I think it is very important for us to remember that we are talking about variants of genes that have been around for a very long time. I don't think there are 'thrifty genes', but I do think there are genotypes – and I am not saying that uricase is one – that under certain circumstances might fit those operational criteria. I am just trying to make a plea for a little bit of clarity in language here.

Richard Johnson: I would call uricase a 'thrifty gene' because the loss of uricase was probably critical for the survival of the species as it is involved in fat storage. In the absence of the mutation none of us would be here. So the mutation was a survival mutation that affected the entire population of apes, great apes and ancestral humans.

Mark Stoneking: I wanted to ask John (Speakman) a different question. In my reading of Neal's formulation it wasn't the thrifty genotype hypothesis – it was a sort of broad scale idea that applied to all humans but there were specific populations to which he thought it particularly applied, including Polynesians. So I was just wondering what your think of the Polynesian as an example of the thrifty genotype?

John Speakman: The Polynesian example is a really strong possibility because we have looked at the genes that come out of the genome-wide association study and they only

explain 2% of that 65% variance in body fat. So there is an awful lot of other variability out there. So one possibility is that there are small populations that have been under intense selection pressures - and the Polynesians are potentially one of these – where there has been gene selection that you could end up calling 'thrifty genes' if you showed that they had an effect on survival probability. The big genome-wide association genes are useless in this arena unless you are talking about genes that have allowed us to survive periods of famine. So, then, coming back to Greg (Wray)'s point, I agree completely that the title of the Neel's paper 'A thrifty genotype rendered detrimental by progress' (42) has actually led to a shorthand version where everybody has subsequently used the term "thrifty genes". The uricase example is a case in point of a specific gene. If Richard could show that the absence of uricase allowed our ancestors 22M years ago to survive putative famine, that would be good evidence that there is a thrifty gene there. I just don't think that evidence exists.

Ingemar Emberg: I am very confused - is there a complete consensus about the 65% impact of genes on our risk of obesity. Secondly given we have an obesity epidemic how can that be 65% determined by genetics?

John Speakman: The 65% number comes from a review paper by Allison in 1995 on the explanations for the variation in body mass index between people in a population (43).

Mark Thomas: Given that the incidences of both obesity and type 2 diabetes have increased since then, surely the only thing is that environmental influences have changed and increased, and therefore the proportion explained by genotype has dropped since then. That would seem a logical conclusion to come to.

Philip James: Actually what we are talking about is a huge environmental change and you have got, presumably, a range of genetic propensities for putting on fat and you pick up more and more people, the more extreme the environmental conditions become. Greg Wray is agreeing with me!

Bill Leonard: The 65% was based on using the very crude body mass index?

John Speakman: Yes and all the genome-wide association studies are based on BMI as well".

Andrew Clark: Trudy Mackay (44) and Larry Harshman (45) and others selected for starvation resistance by taking drosophila flies through a period where they had no food at all. Half of them died, but the survivors reproduced and this was continued for about 15 generations when twice as many survived without any food. The starvation resistant drosophila eat a lot more and put on fat and are able to survive. So when you then put them on just normal food they become really fat and have insulin resistance.

William Mair: With the drosophila selection experiments you also get huge changes in fecundity. Those long-lived flies didn't reproduce much at all and different cross-fertiliser experiments suggested this was not necessarily an intrinsic feature but an absence of reproduction costs.

Bo Angelin: So we need to be broad in our thinking – for example the whole reasoning about the loss of uricase is that presumably it had an evolutionary advantage in very early days as a survival mechanism for some of the great apes a long time ago. Now as humans the interesting consequence is that this makes us more susceptible to sucrose feeding and it may relate also to eating behaviours and perhaps aggressiveness etc.

Philip James: Are the apes surviving simply because they knocked out their uricase?

Mark Thomas: I have no clue but I think the genome is full of variants where there is a difference between us at some level in primates or perhaps in the mammal lineage as a whole so you know you can pick and choose whether you think that that change was essential for our survival. Take another example - the loss of ascorbic acid biosynthesis – is that essential for our survival? Probably not. Most loss of functions relating to the loss of a variety of gene functions simply reflect a relaxation in the selectivity constraints. Now relaxation of selective constraint generally leads to a rapid loss of function because once it

is not being used it just goes into mutational free-fall anyway. If you get a gain of function and it becomes fixed, you have a stronger but not overwhelming case for saying this might be essential – but a loss of function reflects the fact that we have a genome with a large number of lost functions.

Epigenetics and thriftiness.

Anne Molloy: I am wondering though whether it is an epigenetic effect. Thus the yellow and fat Agouti mouse deprived of enough folate end up fat and have diabetes (46). So there is nothing wrong with their genome: it is their epigenome.

Bill Leonard: Correct: but that does not discount the many ways to metabolic thriftiness in humans. The epigenome, responding to developmental acclimatisation in the life history of humans with periods of famine and of food unpredictability can be so stochastic, helping to develop the ability to regulate metabolism and thriftiness over shorter time horizons rather than through longer-term genetic adaptations. These epigenetic changes may be more important metabolically.

Leif Andersson: This responsiveness of the agouti system to folate and other dietary components is not just related as we know to pigmentation but also involves agouti-secreting neurons in the hypothalamic/arcuate appetite control system. This appetite control system in the brain affects the leptin/ MCR4 receptor circuits (47). Over-expression of the agouti signalling protein blocks the MC4R receptor and you get extreme appetite.

John Speakman: Experimental proof of concept doesn't mean that it happened in human evolution.

Mark Thomas: When we think about obesity and diabetes and consider the genetic impact, one of the really big predictors is simply socio-economic class. Now that still belongs in the domain of evolution and evolutionary biology because we live in hierarchical societies and we have done so for at least 10,000 years. But it seems to me that given that that explains so much of the occurrence of obesity and diabetes it should perhaps be discussed in an evolutionary context as well.

Philip James: You mean the social dimension has had an impact on the selection of humans over the last 10,000 years?

Mark Thomas: I don't know – but if you look at the effects of socio-economic class on nutrition, the lower social economic class traditionally are poorly nourished both in terms of semi starvation and bad nutrition with excess calories.

Ingemar Emberg: In terms of short-term adaptation the up regulation of the sirtuin system in yeast allows it to live longer with a delay in reproducing. The same was found in C-elegans and then in mouse models. The animal gets leaner, they live double the time, or at least 50% longer and they postpone sexual activity. So it is a fantastic epigenetic reprogramming, adapting to starvation or famine periods. So this thrifty genotype is ready within days or weeks after exposure to famine.

Bill Leonard: We are seeing that the socio-economic issue is an integral part, especially for humans. I am thinking of some of the work that has come out of Connie Mulligan's lab showing the epigenetic effects of things like poverty, war, marginalisation on many of the genetic systems (48,49). So it is not unreasonable to consider that these larger, socio-economic and political factors are going to have some real trickle-down effects in terms of the parameters that we are interested in.

Philip James: Last time we had a conference we had a real difference of opinion as to the extent of inheritance of epigenetic change so I suddenly thought we were back to major discussions relating to the Russian tradition of adaptive evolution and so forth.

William Mair: As someone who works on the energetics of ageing but also came from a lab that worked on sirtuin and ageing I should mention Linda Partridge's team's paper on this (50) and the emphasis they gave to the non involvement of sirtuin expression in the

control of ageing and lifespan in C-elegans. GWAS scanning in drosophila found very little direct gene association (51). In the mouse it works: if you over-express Sir-2 in the brain then you can increase lifespan, but not by overexpressing Sir-2 in some of the metabolic tissues (52). I think there is a lot of evidence that you can manipulate energetic pathways and modulate lifespan, but the sirtuin research field is evolving with 7 sirtuins being found in mammals with some modulating ageing; there is a clear link between modifying metabolic pathways and the outcome of healthy ageing. But it is not clear that we have one master thrifty recapitulating over-expression of sirtuin system for example that then can affect ageing.

Amanda Henry: When considering the thrifty genotype and ageing we need to realise that in evolutionary terms what matters is reproductive capacity so how much thought is given to maximising reproductive ability rather than increasing lifespan in these studies?

William Mair: I don't think there is a huge difference between lifespan and fitness. I also don't think there is a single dietary intervention that increases lifespan of a model organism without some fitness cost – it is usually growth, reproduction, or something else. So most of the things we do in the lab to modify ageing has some consequence but during evolution obviously populations did not just die of old age.

Bo Angelin: I always thought of the evolutionary pressure as being very simple: you need three things: food that you can assimilate, avoid being someone else's food, and reproduce.

Philip James: And we also heard that you have got to be capable of coping with infections from a crowd phenomenon with cross-infection?

Mitochondrial genetic evolution

Zhenglong Gu: There are hundreds to thousands of copies of mitochondrial DNA (mtDNA) present in each single human cell, in contrast to only two copies of nuclear DNAs. These mtDNAs can differ from each other as the result of inherited or somatic mutations. So although the mitochondrial genome is functionally compact it also shows a lot of difference between populations and most of the major changes will have a big functional consequence. So it is surprising the differences do not show any major phenotypic differences between populations. We know that different aspects of the environment affect this process of mutation accumulation in different tissue mitochondrial DNAs. We recently published analyses of the prevalence of coexisting mtDNA variants in a single cell i.e. heteroplasmic mtDNA in humans (53). We assessed 1,085 human individuals from 14 global populations as sequenced by the 1000 Genomes Project with a mean coverage of about 2,000Å on the mtDNA. We found that about 90% of the individuals carry at least one heteroplasmy and at least 20% of individuals harbour heteroplasmies reported to be implicated in disease. Mitochondrial heteroplasmy tends to show high pathogenicity, and is significantly overrepresented in disease-associated loci. Then we found that when heteroplasmies with an estimated allele frequency larger than 60% were found within an individual without significant pathogenicity, this implied that there had been a purifying effect during natural selection to eliminate those individuals with high hereoplasmy rates that cause pathogenicity.

As people age you have a turnover of mitochondria, so some of these mutations might have a chance to increase in some sub-populations. So we are trying to develop a novel method for high throughput cheap mitochondrial DNA sequencing analysis. Our ultimate goal is to try to sequence 100-500 single cells from each individual in a substantial population to see what the pattern of single cell mutations is. We now have a sequencing method which we are currently applying at a single cell level to look at single cell mtDNA mutation dynamics and how it is associated with future disease potential or current disease status. When the mitochondrial genome mutates it signals through epigenetics pathways via the nuclear genome or other biomolecules e.g. the protein, lipids or ion changes to modify such mechanisms as p53. Unless homeostasis is maintained apoptosis kicks in. This aspect of the research has been handicapped for a long time by the absence of tools for testing mitochondrial genetic changes. We are currently trying to synthesise

mitochondrial DNA. This step is pretty straightforward because it is only 16.5 kb long but the problem then is to deliver this altered mtDNA into the mitochondria itself. We think we have got it now but we are still checking. The goal is to create mutations in the cell line or animal model and try to understand how this process works by synthesising the mutations and then checking to see its metabolic downstream effects

Patrick Stover: I just have a question about the whole mitochondrial DNA story - when you look at nutrient or metabolic responsive genes, there is an incredible amount of buffering that goes on both in terms of methylation patterns and especially at the level of translation when you change the exposure. That buffering then tends to correct the network. If there is heteroplasmy in the mitochondrial genome there must be an awful lot of buffering because you can ramp up the number of either mitochondria or mitochondrial copy numbers. So you would have to have a very penetrant mutation to escape that buffering. Is that true or not?

Zhenglong Gu: I accept that to show an effect is going to be a challenge.

Doug Wallace: This is an integrated system and we should look at mitochondrial function from a systems biology point of view. Heteroplasmies are highly tissue-specific, site-specific, and allele-specific. If you have a mitochondrial DNA mutation, that mutation could have a range of selective biochemical effects. But that effect is modulated by its heteroplasmy so a very severe mutation and a low percentage heteroplasmy will turn out to be neutral but the heteroplasmy has the capacity, through random segregation, to develop to a high frequency and affect the haplo phenotype. In fact Mark Stoneking just had a paper showing that the mitochondrial DNA number of effective copies that are transmitted to the female germline is about 8 (54). So then you can imagine a huge bottleneck with an heteroplasmic cell ovocyte which reduces half a million mitochondrial DNAs to 8 to give the germ line for the next generation. So obviously you can have very rapid fluctuations in phenotypes. So heteroplasmy is important. The nature of the mutation then is also important, so a very mild mutation won't give you an effect unless the cell's mitochondria reflect the pure mutant whereas a very severe mutation can give you an effect when it is at a very low level of heteroplasmy. There are changes in either the control region that regulates heavy strand replication or in the light strand origin. These then affect the bias in the percentage of mitochondrial DNAs. The mitochondria then will change oxidative phosphorylation which in turn changes all the metabolic balances that then affect all the signal transduction systems which then affect the epigenome. So there is a whole range of interactions that are working at both the epigenomic level and on the quantitative biology of impact. So in Leber's hereditary optic neuropathy - a disease that causes blindness – but only does so when it is a pure mutant because it is a relatively mild mutation. Yet in Leber's pedigrees that are, of course, maternal there is a very variable penetrance clinically. Some people go blind whereas other people do not. The really amazing thing is that men are four times more likely to go blind than women, which we also see in the sex difference prevalence of autism. So there is a male/female bias, but then we find other individuals who don't go blind despite having a pure mutation and this is explained by the change in their mitochondrial DNA copy number. So if you have a higher mitochondrial DNA copy number, you are less likely to go blind. However, if you start smoking or you are a heavy drinker, that adds an environmental effect that compensates for the high copy number and causes you to go blind. So we have well-documented interactions – the mutation itself, the copy number and the environment, all of which then affect penetrance (55).

William Mair: So beyond the 37 mitochondrial genes, what are your thoughts on mitochondrial derived peptides such as humanin and other small peptides which can either be translated in the mitochondria or outside of the mitochondria; can maybe communicate between mitochondria and the nucleus and affect metabolic disease? And if you have mutations in those peptides then these can affect the cellular humanin more than the peptide derived from within the mitochondria pool.

Doug Wallace: I would like to just elaborate on these four peptides that occur in the mitochondrial DNA because I think it is a subject that you all might be interested in.

Embedded within the mitochondrial DNA sequence, specifically with the small and the large ribosomal RNA genes, are small open reading frames with a messenger RNA which can only be interpreted in the cytoplasm. Small RNAs are made from the processing of the ribosomal RNA and then the RNA is actually exported into the cytosol and there they are translated on cytosolic ribosomes. The peptides then as metabolic hormones circulate in the body (56). The known peptides originating from the mtDNA, namely humanin and MOTS-c, suggest a larger mitochondrial genetic array than previously thought. These mitochondrial-derived peptides have marked and distinct biological activities with MOTS-c also targeting skeletal muscle with increases in glucose metabolism. So MOTS-c seems to be important in the regulation of obesity, diabetes, exercise, and longevity – a completely new phenomenon. One of the interesting features of MOTS-c is that it is polymorphic and a particular peptide affects longevity in Asians (57). But we have also found that these particular peptides are actually very specifically mutated in certain kinds of genetic diseases. So there are actually accumulating mutations in these particular small regions of the ribosomal RNA.

Reverse migration into Africa

Philip James: In relation to migration whether out of Africa or back into Africa was there a conjoint male and female migration or was it dominated by males moving?

Andrew Clark There actually is completely independent evidence from what Mark Stoneking brought up. Mike Hammer looked at Y-chromosome variance and concluded that it clearly showed this back migration (58).

Mark Thomas: That's not true! Back migration to Africa that Mike Hammer assessed was based on a marker YAP and we showed very clearly that that was not the case – in fact it had originated in Africa and that they had simply got the tree wrong (59)! There are many signatures of migration from outside Africa into Africa but trusting a signature based on the Y-chromosome or mitochondrial DNA alone would be insane because of one very simple principle: patterns of variation in mitochondria and Y-chromosome - being single loci – are only weakly constrained by population history and what that means is they are only weakly informative on population history.

Doug Wallace: I don't disagree that there are issues with single loci – but we can learn a lot about biology by studying a single locus. I often sense there is criticism that there is too much emphasis on the mitochondrial DNA or the Y-chromosome. But that is only true if you want to generalise too broadly. But if you want to know something interesting about sex-determination or if you want to know something interesting about how energetics might have adapted to an environment, then you can study a single locus. I think it is very important not to diminish that information by over-generalising.

Mark Thomas: Please note I was talking about using them as tools for demographic inference only.

Andrew Clark: Differential migration of males and females can be assessed with mitochondrial and Y-chromosome variant analyses. Also you can get at that from cultural anthropology, just looking at how much movement do you see of males and females; female migration was predominantly local migration.

Mark Thomas: More societies today are primarily patrilocal – but that doesn't necessarily say that human societies in the Pleistocene era were more patrilocal – although I suspect they probably were to a large extent.

Mitochondrial DNA analyses and metabolic variation

Philip James*:* We've had a great discussion about the evolutionary pressures and the movement of people and the way in which that happened. We have also mentioned mitochondrial DNA changes. Sarah Tishkoff has described a whole array of people in different environments and different dimensions of regulatory metabolic processing in Africa but where have we got to with mitochondrial typing in different societies within

Africa? If mutation rates are so much higher in mitochondria than in nuclear DNA are we seeing extraordinary different metabolic responses in different African societies?

Sarah Tishkoff: Mitochondrial DNA has been extensively studied in Africa but we have been looking at it as a tool to reconstruct evolutionary history, not looking at it in terms of function and integrating it with the nuclear genetic data and the phenotypic data that we are getting.

Doug Wallace: We did the first survey of mitochondrial DNA in Africa, published in the early '90s. We defined all the major lineages and we actually did quite a lot of field studies in Africa at that time. So we have the first collection of Khoisan for instance in South Africa (60) and I need to talk to Sarah about using some of these data in relation to her interest. We found a number of major lineages and those lineages have very unique genetic variants. Some of them look potentially relevant physiologically. For example we have taken the major lineage L, which relates to all Africa and compared L with the most common lineage in Europe, haplotype H, and then made what we call cytoplasmic hybrids where we have just the mitochondrial DNA with a standard nuclear background: the RNA transcriptome then shows huge differences. Specifically L operates to guide much of the NAD immunity genes relevant in Europe. So that raises the possibility that there is some mitochondrial physiological component to factors affecting infection. But all of this is very new and it takes a lot of time to do these experiments.

Zhenglong Gu: It is still very difficult to insert DNA into the mitochondria to test their selective effects.– people have been trying for at least 3 decades or more to deliver the mitochondrial DNA into mitochondria.

Philip James*:* You imply that these mitochondrial changes have profound physiological effects but are these just seen in cellular studies rather than in whole body physiological variables?

Doug Wallace: We showed a very clear difference in the mitochondrial DNA lineages in Northern Siberia versus Central Europe. Then people have actually looked at the BMR of these two populations and there is about a 25 to 30% higher basal metabolic rate in the far Northern populations relative to Europeans and that would then be consistent with the idea that there are less efficient mitochondria with more uncoupling and that would generate more heat.

Philip James*:* Wow! As somebody who has spent my life measuring metabolic rate I didn't believe that anybody's metabolic rate unless it is measured under the most meticulous conditions with standardising for fat free mass – in other words lean tissue. Once we did that then we found the variation within a single population was really quite limited

Bill Leonard: Our group did that research on basal metabolism in Siberia. All our work is standardised for fat free mass and what we find is that those elevations are consistent with what we see in Inuit populations as well – an elevation of 15-20% above what would be predicted based on fat free mass in both men and women (61).

Mark Thomas: I want to ask a question here – assuming that you got those metabolic differences, I am worried we are getting the waters muddied a little bit here. Were these differences measured in individuals with different mitochondrial haplogroups or at the population level of comparison? If it is at the population level of comparison, well there are an almost infinite number of explanations for that; but if it is relating to differences for specific haplogroups then you that is something more interesting.

Doug Wallace: I don't know!

Bill Leonard: We have run the analyses both ways basically, so we have haplogroups variation and so within the haplogroups that Doug talks about as having the greater uncoupling, we do indeed find that those BMRs are elevated above standards. What we are currently investigating is the degree to which brown fat may also play a role.

Philip James*:* We worked on brown fat 45 years ago, despite the recent literature ignoring it (62)! We had to look back in the literature and there were beautiful studies actually

coming from Finland and so on showing that brown fat and its remarkable physiological adaptations to environmental conditions in Arctic peoples but I have no idea what their haplotype was. But the physiological adaptation is quite substantial on a seasonal basis. There have also been beautiful studies of patients with phaeochromocytoma where there is also a proliferation of brown fat (63).

Bill Leonard: I have a doctoral student who is just writing this up. She is using thermal imaging as a way of quantifying brown activity after a cold shower! We measure our subjects under basal conditions, then expose them to a cold shower and look at changes in metabolic rate and the presence or absence of brown fat. What she finds is that greater levels of brown fat from the thermal images are associated with significantly greater rises in metabolic rate in response to the cold shower.

Philip James*:* But if you take out the brown fat component, do you find in haplotype terms that the rest of the non-brown fat tissues are less efficient?

Bill Leonard: That's a question that we are still exploring.

Doug Wallace: What would be the significance of a mitochondrial DNA variant? It is not going to be the kind of short-term variation that you talked about. That's going to be regulated by the circadian rhythm, by annual variation and these are going to be regulated by nuclear genes. So the uncoupling protein 1 in brown fat is an inducible gene. So clearly there are physiological ways to deal with acute environmental changes. Those are going to be primarily nuclear. What we are looking at is deep time evolutionary adaptation that sets the upper and lower limits. So there is no conflict between the wonderful thermogenic studies on brown fat and the mitochondrial issues. They are looking at different timeframes.

Sarah Tishkoff: It is not easy to do these types of study in the African bush. We look at the genomic, epigenomic, RNA and metabolomics and try to integrate those together (64). So the metabolomics give you a snapshot of the physiology and provides some basal data but it would be interesting to then have a challenge of some sort and see what happens. In Cameroon we are going to get people to wear a wristband and measure their heart rate and activity, waking and sleeping cycles and how much movement and running around they are doing, and then we sample these data every day. Then we will look at changes in gene expression and correlate these with more physiological measures.

Philip James*:* When you see differences in the metabolomics data are you distinguishing between the impact of different diets or of the types of mitochondrial haplotype?

Sarah Tishkoff: We need to look at the impact of the mitochondrial but we cannot ignore the nuclear DNA! That is one of the ways we hope to control for ancestry. So let's say you could take two groups that have the same genetic ancestry, but now with a very different diet or living in a very different environment. Fine but then you can do the reverse analysis by looking at say two groups living in exactly the same environment and perhaps on the same diet, but where they have very different ancestries. That is one of the ways we will try to figure out the distinction between genes and the environment.

Anne Molloy: We are working on the role of folic acid in the prevention of neural tube defects (NTDs). We looked for candidate genes and took normal populations and assessed them by GWAS scanning in relation to the one-carbon metabolites. It is actually very difficult to find something that is really a driver or an important determinant of NTDs, despite the fact that we know that folic acid prevents NTDs. But the genes that are involved in mitochondrial metabolism and the provision of one-carbon units and of formate need assessing. Secondly our genome-wide association study on a very homogenous group of students in Ireland show that the metabolites that you look at in blood simply reflect the transport proteins that allow their passage into the blood stream. So these blood changes may not correspond to the intracellular events.

Greg Wray: To answer your question how much is genotype and how much is environment is, generally speaking, an extraordinarily difficult thing to do in human beings. If you take individuals and give them a different diet or exercise them you still have the epigenetic

imprint of the prior weeks, months, maybe even years of previous lifestyle, diet etc. effects. We just don't know how to disentangle that from the short-term manipulations that we are assessing.

Richard Johnson: We have been interested in the last few years in these big epidemics of chronic kidney disease occurring in very hot regions of the world. So far we have analysed 50-60 thousand deaths in Central America, India and Sri Lanka linking them to heat stress and particularly with increasing core body temperatures. A very interesting finding is that the African population seems to be protected, whereas Hispanics are not. This may relate to the mitochondrial uncoupling theory proposed by Doug Wallace. So do the Hispanics carry more native American genes that might be more likely to carry the mitochondrial clades associated with uncoupling? The evidence suggests that the core body temperature is going to have a major role in regulating the effects of heat stress and climate change. We have actually linked the energy uncoupling to the development of obesity and metabolic syndrome through a pathway that involves vasopressin and, of course, fructose (65).

Microbial metabolism

Ingemar Emberg: We have just had a conference at the Karolinska on the microbiome with Geoff Gordon and other leaders. They have shown, pretty convincingly, that the gut microflora is an integrated part of the human genome activity and physiology. The numbers vary, but between 10% and 20% of the metabolites in blood come from the microbes not human cells. So how can one design studies to distinguish the different components relating to the nuclear genome, the mitochondrial genome and the microbiome?

William Mair: You have the microbiome interaction with the metabolites but we have been looking at steady state metabolite levels – it doesn't tell you much about the important issue of flux but these are hard to document in humans. Then there is the circadian component – mice studies show that the status of the mitochondrial network, whether it is a fused or fragmented mitochondrial effect, affects the capacity to metabolize nutrients. So I would caution against over-interpreting the significance of blood metabolite levels if you don't know whether they are human derived or bacterial in origin.

Mark Thomas: I just want to make a general point about microbiome research – I wouldn't confuse lack of progress with lack of interest. There are few areas in biological sciences more fashionable than the microbiome! Perhaps maybe epigenetics, if you are lucky! I think in general progress is pretty much dependent on the amount of interest divided by the complexity of the problem! (*Audience laughter*).

Hannelore Daniel: I think this microbiome world is in publicity terms overdone. So we should go for better phenotyping approaches and recognise that human metabolism is very dynamic but also has enormous buffering capacity. So we need to develop challenging experiments with standardised approaches.

William Mair: Mark Thomas noted that two of the coolest things in science are epigenetics and the microbiome but the missing sexy piece now is CRISPR. We now have a massive capacity to assess the combined effects of different SNPs on function by genome-editing all in one go. So I don't think we are going to find strong effects linked to a single SNP mutation– it's going to be communal effect. So with a high throughput method using model organisms rather than mice we can make 40, 50, or100 edits in one go and then look at the effect of these changes on their interaction with diet. We heard yesterday that some of the mechanisms you see and their nutrient interactions are conserved across the species and even in drosophila and other species.

Philip James*:* OK but if one measures human metabolism under extremely accurately controlled conditions e.g. in metabolic terms then one can see reproducibilities of 1%. Then one can change the environmental conditions under very tight control and assess the effects and indeed discriminate the differences between individuals in their seemingly

"intrinsic" responses. So it is going to be a challenge assessing intrinsic population differences in metabolism and their basis.

Andrew Clark: I absolutely agree it is possible to measure things with considerable precision in individuals, but you have to realise that each individual is a realisation of a genotype going through a whole series of environments in their lifetime, and that is unique. To disentangle these effects you do factorial experiments, where you take a bunch of genotypes and a bunch of environments. We do this with humans and we do it with model organisms all the time. In model organisms we can identify the fact that there are often interactions i.e. a genotype/environment (G by E) interaction. So if you try to look for a G by E in humans, and you know they don't have the same genotype you can assess the interactions of single SNPs when the environment is different e.g. on different diets. But it is extremely hard to find any evidence for G by E in humans. So the reliance on model organisms is really important to gain an understanding of potential mechanisms.

Population differences and the impact of increasing life-spans

Rolf Hultcrantz: The medium life span for human beings was as much as 50 years until 1850 but since then it has just gone up and up. Now it is about 85. That means that genes that were vital during all these earlier millennia may be dangerous these days. For instance, I work with haemochromatosis and that is a fantastic gene if you are a woman, with a mutation and give birth to perhaps five or six kids then you were unlikely to die of this condition. But now it is very dangerous mutation in older people. Another example is the genetic variation in patatin-like phospholipase domain-containing 3 (PNPLA3) which confers susceptibility to non-alcoholic fatty liver disease which makes you more susceptible to the currently common liver cirrhosis unrelated to alcohol drinking (66). So a lot of this environment stuff combined with age gives us a completely different picture of diseases and nutrition that is more important than the microbiome.

Bill Leonard: I want to remind us all that many of these questions have been looked at in the classic human environmental physiology literature on adaptation in the first half of the 20th century. We saw a striking and meaningful geographic variation across human populations in aspects of basal metabolic rate, tolerance to heat and cold, maximal working capacity, the classic work of high altitude physiology – these studies were all looking at the important human variations but there was not the capacity to look at the underlying genetic signatures. So this is where our lessons from high altitude adaptation I think are very telling. The classic work by the end of the 1960s came to the conclusion that genetics have no role in high altitude adaptation. We now know clearly that not only is that not the case, but that different high altitude populations from different parts of the world adapt to high altitude in different ways. There are different genetic signatures so natural selection had impacts in different ways. Indeed there are different features of people's physiology in the Andes compared with those observed in the Himalayas or in African high altitude populations. I think that this should be a cause for optimism - we can begin to pull these genetic and larger functional physiological dimensions together in a more integrated fashion.

David Rosenblatt: If we look at the problem of haemochromatosis we used to think that when we knew the gene for haemochromatosis, we would do large scale population screening and then pick up all the people who have the gene and then therapeutically intervene. But when the gene was found, it was clear that it was highly inefficient because patients with haemochromatosis who were referred from hepatologists all had liver disease. But in the general population very few people who are homozygous for the gene for haemochromatosis actually get the disease. So generalising on the basis of SNPs is useless. Until we are in a position when we can do whole genome analyses at one time on a large population, we are never going to know what the genetic contribution is in particular circumstances.

Tommy Olsson: I completely agree on the approach to deciding when to intervene but I think we must also try to understand why certain interventions work with certain ethnic backgrounds, with gender and age differences etc. Phenotyping is also important and when

done in parallel it can help our understanding of ethnic differences relating to environmental pressures.

Anne Molloy: I think we should also consider the likelihood of having multiple genes with low penetrance accounting for nutrient-related chronic diseases.

William Mair: I work on a model system – C. elegans, a nematode worm. We work on nutrient responses to physiology and healthy aging. These worms are isogenic, i.e. clones of each other. Now if you age them over about 3 weeks some of them age very rapidly and die within 12 days and some of them age slowly and die in 25 days – so you get 100% difference in response to longevity and aging in an animal that has got exactly the same genome under identical environmental conditions. Then with nutritional changes you can modify the lifespan of these animals and find different responses in different individuals to nutrients when they have exactly the same genome. So we should indeed be thinking beyond the issue of whether we have SNPs singly or in combination - clearly an epigenetic or stochastic change alters the response of animals to the way they interact with their diet.

Zhenglong Gu: But with C. elegans it is very difficult to assess their copy number before they are dead. We look at young drosophila and find huge differences in the isogenic flies; we find there may be a big difference in terms of copy numbers.

Richard Johnson: I think one of the key issues is defining the phenotype. So, for example, some people would like to consider hypertension as a disease state but actually, hypertension is a manifestation of systemic vasoconstriction along with normal cardiac output: multiple mechanisms drive vasoconstriction only some of which may actually be evident depending on the environment or other circumstances as well as the effects of selective drugs. Similarly the PNPLA3 gene causing fatty liver depends on alcohol drinking for its worst effects and the same environmental issues apply to the haemochromatosis gene.

Rolf Hultcrantz: About 20% in a post-mortem survey were found to have a fatty liver but suddenly about 25 years ago this turned into a disease when people put on weight. I have hundreds of these patients with inflammation; fibrosis etc. and you end up needing to do a liver transplant. This was not seen 30 years ago. So something has happened. Those with fibrosis have the mutation brain nutrient peptide (bnp03) and those who also get liver cirrhosis from drinking also have that mutation (67). The environmental change is the critical feature as the prevalence of the genes is exactly the same.

Jere Haas: 40 years ago we looked at differences in adaptation around the world but we didn't have the luxury of these new genetic analyses. But I want to emphasise that we shouldn't now say that we can explain these population differences strictly on the genetic variation. A lot has to do with epigenetics. When I was trying to look for opportunities for natural selection in populations at high altitude, we looked at pregnancy. Now Roberto Frisancho in the early '70s assessed what he called developmental adaptation (68). It implied that exposure to the environment at a critical stage, generally very early in life, had conferred later advantages. This adaptation occurred in populations that were genetically very similar but had different early exposures i.e. early experiences at high altitude. So I think that is a model that ought to be considered when we are starting to look at the population level using the genetic differences that we now are able to determine, but also recognising that they actually may or may not be responsible for some of the developmental changes that confer adaptation or advantages in populations.

David Rosenblatt: When we consider why diseases are different today than they were a generation ago – some of the effects may relate to immunity. For example people assume that streptococcal infections and rheumatic fever are now much more infrequent because of the advent of penicillin but it is not penicillin and treatment that has explained this change. I don't know what has made it go away. People now throw in "epigenetics" when faced with any conundrum.

Philip James*:* When we discussed this only a couple of years ago it was very clear that epigenomic scanning was becoming a major endeavour. It also becoming very clear that

only when you have put together the epigenetics and the genetic scans can you then begin to get much better explanations for why there are physiological abnormalities and disease.

Mark Thomas: Epigenetics is very fashionable and it deals with a beautiful and amazing set of mechanisms that warrant a lot of scientific study. It is also almost certainly important in a variety of circumstances. The other thing that highlights epigenetics is the development of bisulphite sequencing. We have a methodology so it makes it much easier to study, but let's not forget the fact that unlike the central dogma of molecular biology, epigenetics opens up a lot of potential complexity. Our job is to test hypotheses and it is easier to test simple hypotheses than complex hypotheses, so let's not confuse simple explanations being more right when we should be prepared to look at more complex things.

Dietary impact on gene selection.

Patrick Stover: The topic of this symposium is nutrition and genomic evolution. A lot of us study, or try to model in mice or different organisms genetic variation that sensitises you to different environmental exposure. But when you try to design these models to create genetic disruption or genetic variation, you often see the mice compensate. There is a buffering capacity in these networks so they seem to read the endpoint of the network and then try to re-establish network function through changing gene expression or translation. These epigenetic and other effects of genes all buffer these network outputs based on what their genetic architecture is. So when you look specifically at how nutrition has driven genome evolution to what degree in practice has nutrition played a role in changing the genetic architecture? There seems to be positive selection in the haemochromatosis genes and we know that lactase reflects positive selection that may be related again to infection more than it is related to the food source. But other than that, how many examples do we have where nutrition as an exposure has changed the genetic architecture such that it is beyond the buffering capacity and you are in another space?

Sarah Tishkoff: Part of the issue has to do with the complexity of the traits. So one of the reasons we don't know the answer is that there are complex traits – it is difficult to identify what the genes are that are playing a role. But one way we can address this is by looking at either individuals or sometimes populations that have extreme phenotypes. Those extreme phenotypes may be adaptations to different diets or different environments. For example we are studying pygmy populations in Cameroon. Now not only are they extremely short, but there are alterations to their growth hormone IGF1 axis that is influencing insulin metabolism and also lipid metabolism. We are seeing differences also in the pituitary/hypothalamic thyroid related functions. So one idea that I think Mark Thomas here proposed a long time ago is that these populations nowadays are living near agricultural groups like the Bantu but they moved in there only in the past 5000 years. Pygmies had, however, been in their traditional habitat for 40,000 years or more. Now we find that the Pygmies don't get goitre whereas the neighbouring groups do. One theory is that the Pygmies have been adapting to a low iodine environment which induced some alterations to thyroid function, whereas the Bantus and others did not. So the Bantus are now susceptible to goitre in their low iodine environment. I think that by looking at groups over long periods of evolutionary history that allow adaptation, we may get a clue to these adaptations.

The change in genetic architecture is still an outstanding question. In dogs one often sees mutations in the growth hormone/ IGF1 pathway and it turns out that in Pygmy populations all over the world – whether they are in the Philippines, Papua New Guinea and probably in Indonesia all show alterations to growth hormone and IGF1 pathways. So why in all these Pygmy populations do you get independently alterations to that pathway? That pathway influences many aspects of physiology and so it could be that it has to do with diet or reproduction or something else.

Mark Thomas: I agree completely with Sarah but there are quite a few other hypotheses. We have got a lot of the low hanging fruit when it comes to signature selection: you have lactase and probably amylase but the jury is still out about whether the copy number variation in amylase is the key and how important that has been in recent evolution rather

than maybe over the last 400,000 years since the invention of fire. There are other ones: there are lipases to do with lipids in plants; there is some evidence relating to folate metabolism.

Patrick Stover: There are genes in folate metabolism that have a profound effect on both the accumulation of folate and its flux but none of them show signatures of positive selection.

Mark Thomas: OK but my understanding is that in terms of folate biosynthesis there is some evidence of selection in some populations. It is all a question of your sensitivity in your tests of selection. Unless you go the more interesting route that Sarah mentioned which is the multi-genic approach where you have a clear phenotype that you are interested in and then you are stuck with whatever GWAS hits come up and then you look at those *en masse.* This is a neat approach that has been used recently for height, for example. But at the individual gene level, one's power is limited using the methods that are based on modern genetic data. The most sensitive to change over the last 30,000 years are the haplotype-based schemes. I think that our analytical power is going to be increased quite dramatically as we accumulate more ancient genomes because then you can measure time point A, time point B and in between, and you can see those allelic gene change trajectories. Plus you have local knowledge of potential ecological drivers of that selection and that also helps in the mix when testing hypotheses of selection. But there are others around as well. You know a classic one is the alanine glycoxylate amino transferase which is involved in oxalate kidney stones and so on. There is a clear preference for alleles associated with either a more vegetarian or a more meat based diet and some evidence of selection – not strong at the moment, but potentially there. I think the answers are going to come mostly from ancient DNA over the next 10 years and I think we are going to be very happy with the results!

Greg Wray: I agree with Mark that absence of evidence is not evidence of absence – a lot of our tests for selection are pretty underpowered. We are also assuming that these issues relate to large effect monogenic traits and they do not. I think we will have a lot more sets of evolutionary changes that will partly come from analyses of ancient DNA, but also from having a better handle on the genetic architecture.

Bo Angelin: Cynically you could say that human evolution can be explained to a large extent by rape and murder! If you are a conquering population, it is very important to mix as soon as possible with the people who may have those protective features they have developed and make it easier to survive in that region.

We were also thinking many years ago about pygmies. Growth hormone obviously has a powerful effect on the regulation of lipid metabolism and you can actually explain part of the ageing effect on cholesterol metabolism by the fact that growth hormone secretion is going down with age and you have differences between males and females. You also find in the old epidemiological data the claim that relative body weight or BMI is a risk factor for coronary heart disease when actually it is not overweight but a short height. If you look at the basic data, people that develop early coronary heart disease are actually shorter than the others, not fatter. – I mean on a population basis. And that has been explained, of course, by claiming that if you are small, you have a Napoleonic complex which makes you respond when stressed! But it could also be that by secreting more growth hormone gives you an advantage in terms of fatty acid release etc.

You also have this stochastic effect. I think the C elegans data is very beautiful, but when we have complete knowledge of everything we know exists including all the latest omic data the functional outcome will also be a question of luck. That is very good because we will never be able to predict the effect when it comes to outcomes disease development in the individual because there is a pronounced stochastic effect and there is no gene as far as I know for luck!

Anne Molloy: We as biochemists and nutritionists try to push the idea of gene selection relating to our favourite polymorphisms and that certainly has been done for the methylynetetrahydrofolate reductase (MTHFR) folate polymorphism. This is a very well

described functional polymorphism with disease associations and markedly different prevalences worldwide: low prevalences among African populations but very high prevalences among Mexicans. Prevalences are quite low in Northern populations. So some of the first studies claimed marked selective pressures with the gene surviving in Mexico and in Sicily where there is a very high folate status. Then you go to China and the reverse is true with people there with the highest prevalence of the TC genotype despite having the lowest folate status. So I think a lot of us don't accept the fact that the genotype is there and we live with it and then the disease risks etc. are the consequences of these interactions.

A second point and perhaps more interesting finding is that when you do a GWAS relating to vitamin B_{12} what pops up as the most significant factor is the FUT 2 that relates to your secretor status and has nothing to do with B_{12}. It is more likely that the secretor status is impacting on nutrient status. So the FUT2 secretor variant worsens B_{12} status in cases with heterozygous gastric intrinsic factor mutations which then affect the absorption of B_{12} (69).

Brian Fowler: If we focus on SNPs in relation to a metabolic pathway then we have to recognise that a single SNP does not work in isolation but as part of a pathway. With the MTHFR, there are some correlations in certain populations and certain sub-groups, and it is not easy to interpret because obviously the SNPs reflect the generation of a protein but proteins work in association with other proteins. So we need to look at the whole profile of SNPs. Sarah is now doing metabolomics which is an excellent approach even if the use of single fasting sample has limitations.

John Speakman: For a long time the limiting factor when assessing any relationship between genotype and phenotype was the cost of doing the genotyping. Now the cost has dropped spectacularly so we can generate lots of genotype information for individuals. But the problem is that the phenotype specification that we then try and associate with the gene profile characterization is not improving at the same rate and in terms of quality.

Stephen Simpson: I agree we need to define the phenotype carefully. We also need to define nutritional interactions as taking the single nutrient approach has the same limitations as single SNP approaches.

Vimundur Gudnason: I come from a scientific background where we have very large data sets on populations with both phenotypes and genotypes being assessed. It is important to harmonize the phenotypes and make sure that when you are doing all these analyses of genes you can replicate the evidence because there is so much multiple testing which reveals that a finding obtained after a huge amount of work cannot be replicated. So it is extremely important to be accurate in phenotyping and to make sure that you have the capacity to replicate what you are finding.

Sarah Tishkoff: You need to keep in mind that one of the problems when you do genome-wide association studies is that it is hard to get a significant p-value. In some ways I think we should just look to see if there is a correlation in a pathway or metabolic pathways

Lorraine Brennan: I just want to emphasise the limitations of using fasting samples when assessing metabolomics: we need to challenge the system with standardised challenge tests: mixed meals, high fat meals, high glucose loads – this will display the phenotype.

David Rosenblatt: Just a note of caution on model organisms for which I am a strong advocate. We are burdened with inherited metabolic disease with a fairly mild phenotype in the human but when trying to reproduce this in the mouse this reveals a severe disease or conversely you have a severe disease in the human but no phenotype in the mouse. So you just have to be very careful in your generalisations.

Doug Wallace: One of the problems is we may not have the appropriate tests for the things that we want to study. So we have been challenged by the question - how do you evaluate mitochondrial energetics? We don't have any direct tests for that, so we have been trying to develop a number of approaches. One of them is near-infrared spectroscopy as a way of looking at mitochondrial function (70). Some classic ones are p-

31 phosphorus imaging – but these are very tedious and indirect methods. So if we really want to understand energetics, we need to have a test that can be used on individuals in the field and right now we don't have that.

Changes in the food chain and population differences in food needs?

Mark Thomas: Early agriculture developed about 11 to 12,000 years ago in the Middle East and it entered Europe 8,500 to 9000 years ago. It took longer to get into India so most major domesticates in India were relatively late.

Clark Larsen: I would argue though that 3 or 4 or 5000 years in terms of the whole human evolution – which is what we are talking about here – it is just a drop in the bucket and we need to think to go back 300,000 years or more.

Mark Thomas: There is no reason whatsoever to set the clock ticking at 200,000, 300,000 or any particular point – that is an obsession of paleoanthropologists

David Rosenblatt: There are many examples of decisions made by humans which affected food systems - for example the introduction of moose to Newfoundland, or rabbits to Australia. So we need to question whether an analysis of human evolution informs us in any practical way about food policies with respect to nutrition.

Philip James. During this last century we have seen huge changes in the food system. During the 2nd World War the war – time experience of the UK with its food rationing system proved exceptionally successful. It was based on new nutritional concepts emphasising animal proteins then aimed at stimulating stunted children's growth and the need for energy rich foods such as milk, butter, animal fats and sugars. So it became the policy not only for the UK but for the whole of Western agricultural policies to prioritise these foods. From then on meat production, milk production and fat, oils and sugar production were subsidised and promoted throughout the world and it became the global food policy. So in the last 80 years we have seen a transformation of the whole of the food system which is increasingly affecting most countries in the world. But the assumption in policy making for changing the food systems of the world is that different populations have the same intrinsic needs for different nutrients

Bill Leonard: I agree that the underlying assumption is a conservative one: that all humans are essentially the same and that is – from what we have been hearing – in conflict with our growing understanding of the biological and nutritional diversity across humanity. Now how do we begin to reconcile that? Well, there is a growing area of scholarship called 'Evolutionary medicine' - Randy Nesse (71) has been a leader in this attempt to try to work at how dimensions of evolutionary theory can be incorporated into our understanding of this mismatch between contemporary urban society and what was once adaptive in the past. Essentially James Neel started this with his thrifty gene model and was really the initiating of that kind of thinking. So I think an incorporation of evolutionary perspectives can have some real salience in developing policies.

Mark Thomas: We are primarily interested here in the relationship between diet and health. So traditionally there have been two types of approaches to that: one is the kind of bottom up, understanding the biochemistry, understanding the molecular processes that are going on. It is a fantastic approach, lovely science-type of approach, but fundamentally we have only scratched the surface of the levels of molecular complexity that are really at play, at least in terms of the relationship between diet and health. Then there is the kind of top down epidemiological approach. But fundamentally there are always confounding variables and that will always limit the use of those kind of approaches unless we really do extremely expensive types of approaches. So this is why I would argue that evolution is a third way: it is not radical, it is not alternative to these approaches, but what it is predicated on is a very simple principle which is, to a first order of approximation, our metabolism should be optimised to the diet that we have experienced during our evolution. So that puts as a priority understanding what exactly that diet was. Then there are the caveats which are to what extent have we adapted to changes in the diet since that long period of Palaeolithic diet?. I think the problem is that current advocates of the Palaeo diet people –

and most people would probably agree that it is fairly faddish! – is that they do not address this underlying question of what did we eat? What is the extent of diversity of what we have eaten in the past, and without knowing that we can't really even apply this broad general principle of trying to link potential evolutionary changes to optimise our responses to these diets.

Amanda Henry: I agree that to try to assign one set of standards in terms of health policy can actually be detrimental to certain groups of people. So accessing information about the diet that we ate in the past is extremely difficult, but could be informative.

Stephen Simpson: I think we also need to add ecology into that perspective because fundamentally it is the relationship between our evolved physiological mechanisms and our ecological circumstances that leads to the outcomes that ultimately manifest in health. So we really do need to define the current nutritional environment and to ask questions such as, what is a balanced diet? We don't even know how that balance of nutrient needs changes during the life of an individual. How also does it vary from individual to individual? How does it change with their circumstances? What is it that is fundamentally human about those relationships and how do individuals differ and how does that difference or set of differences or variations relate back to evolutionary history? That is where you get the practical advice to give to medical practitioners.

Richard Johnson: Our group for the last 10 years has been adapting Steve Benner's concept of 'planetary biology' (72) which considers multiple approaches – not just evolutionary biology, physiology, molecular biology and genetics, but also ecology history and comparative physiology.

David Rosenblatt: I am fairly naïve in terms of evolutionary science, but thinking as a geneticist – were these diets in place to allow people to reproduce or to live a long healthy life? So it may be good for keeping the species alive but what is it going to do for the individuals? What age end point are we aiming at - do we want this for people to live for 3 score years and ten or are we aiming at 100 years? Are we looking at the survival of the species, or the health of individuals?

Bo Angelin: If we think that there was a Palaeolithic diet to which people were adapted that doesn't say that that is what we should now eat. I think that adaptation was driven by the factors affecting the survival of the species, not individual health or well-being. There is relatively good evidence that our real forefathers actually ate each other as with other animals. They were also running miles a day, often fleeing from danger and constantly stressed by trying to catch foods on the one hand, and on the other hand trying to avoid being captured by animals or foes. So if we want to say that we should have the diet that they had, we should also live the life they lived! And I think that is definitely much more difficult to advocate because there is a difference between exercising three times a week and actually running for your life on many occasions throughout your lifespan. .

Kaare Norum: I would like to focus on the last 50 to 70 years and consider some examples from Norway. We had the German occupation from 1940 to 1945 and there was very, very little food for the Norwegian population because the German soldiers took all the food. Yet in 1944-45 the health situation of the Norwegian population was remarkably good! We had very little food, no cars, so we were very healthy during that period. Just after the war we get lots of food again and then the rates of coronary heart disease exploded in just 5 years. Then over the last 70 years we have worked out the very different types of lifestyle that have a tremendous impact on health and welfare and these conclusions have determined Norwegian food and nutrition policy which we also used for WHO policy making too.

William Mair: The genes which are optimizing our fitness are also the ones which make us age. We have added 35 or 40 years to life expectancy over the last century through public health measures including sanitation, education and antibiotics. So when we are trying to think about how we are going to optimize diets and find nutritional interventions for health, we have got to realise that all the diseases we are trying to cure now are things which are being exposed because we are surviving way beyond the point that we were evolved to cope with. So then the nutritional interventions that you need are not going to be the ones

that optimise fitness. I am not saying we don't use evolution biology to inform nutritional understanding but we are in very different circumstances due to public health in the last 100 years than we were ever in the course of modern human evolution.

***Philip James**:* I see a lot of nodding of heads round the tables.

Stephen Simpson: We can try to optimise foods, menus and dietary patterns to help living healthily into old age or it could be having a maximum reproductive output or recovering from surgery. But what we need is to start to map those topologies so you can take human data from cohort studies or national surveys and you can start mapping topologies and associating nutritional factors and outcomes –that really is what you need to have precision nutrition interventions that will ultimately have public health benefits.

John Speakman: I agree we probably optimised reproduction rather than anything else. But that is not a prescription for what we should be trying to do now.

Patrick Stover: In 3-4 weeks' time the National Academy of Sciences in the US is going to issue a report where the Federal Government is now agreeing that diet is related to the prevalence of chronic disease (73). Since we cannot nationally afford to have these chronic diseases we must therefore stop setting recommended dietary requirements which affect the whole food system unless these are based on preventing on reducing the prevalence of chronic disease. So the problem that we have now in setting dietary recommendations for chronic disease, rather than for function, is that in the population you have responders and non-responders to particular foods or nutrients. So you have people who respond differently to diet but also there are very variable intakes. So you have to begin to classify who is a responder, who is a non-responder with respect to both the dietary exposure and then the risk to that disease. Then the question is to what degree does evolutionary biology help us classify individuals as responders and non-responders, both on the end point of chronic disease as well as on the exposure side.

***Philip James**:* Medics have always considered the individual clinically. But we are dealing with 7 billion people in the world and we already know that there are individuals within a population who respond remarkably differently. But the big issue for WHO is do we think of each population as the same? So far we have been producing for the World Health Organization evidence based mostly on European and American studies of supposed dietary measures with chronic disease outcomes and applying this to all populations as well as ignoring the well known individual responsiveness.

Jere Haas: Keep in mind that half the world still lives in conditions far worse than the ones that we are living in right now. We have this dual burden of malnutrition with undernutrition relating to insufficient food and nutrients and then the problems that lead to chronic disease with obesity. It is a totally different context from the developed world. We have to think of the evolutionary implications of dietary recommendations for probably half the world with inadequate nourishment.

Mark Thomas: When considering the differences between different populations I think evolutionary biology does speak directly to that question. First we can set the base line: the base line for population genetic differences is about 10% of total genetic related variation and 90% is just due to differences between individuals within populations. So we are starting with a relatively small proportion but if you look at the genetic variance that has been under selective pressure, then a lot more of the variation is explained by differences between populations. This is, of course, due to local adaptation. So then it becomes much more important to understand those local adaptations in order to understand the underlying basis of differences between global populations in their response to different medical and dietary regimes.

***Philip James**:* I was involved with a study in Asia where we found they have a bigger propensity to diabetes when they put on weight compared with Australasians – there is a two to five fold increased risk of diabetes at each increment of BMI and/or increase in waist circumference (74). And that seems to be so throughout Asia, although there is an element where the Chinese may be somewhat less susceptible than the Indians. The big issue

then is whether this is environmentally determined e.g. by malnutrition in early childhood or where maternal nutrition has altered the fundamental physiological systems of the body so that people are then 'programmed' throughout their life history for a greater propensity to these problems. We then went on to show that in Mexico, using the huge national database compared with nationally representative data of US non-Hispanic whites that Mexicans are also 2-4 times more liable to diabetes on weight gain (75). Now we are about to publish, with WHO in the Middle East, that the same applies to all the Middle East populations where they have 2-3 times the diabetes rate for each excess increment of weight. In other words, we are getting to the point where we need to ask the question of whether the Caucasians who have lived in Europe or went to America for the last two to three hundred years are a genetically selective sub-group? Yet we have been making global policy on the basis of our European and North America data. That is the dilemma.

Bill Leonard: Exactly: I think you have hit on a critical element – it gets back to the point that Jere was talking about in terms of chronic disease throughout much of the world, particularly in the developing, low income countries. I think there is clear evidence for both undernutrition and overnutrition creating a dual burden. Is that early life undernutrition, then, essentially setting up adults for overnutrition and metabolic diseases later in life? That, I think, is going to have implications for different kinds of policies to address these problems in different parts of the world.

Brian Fowler: I would like to home in on one specific example of nutritional intervention and that is the fortification of food with folic acid. There are many issues around this. One primary aim is the prevention of neural tube defects. But attached to that was the idea that homocysteine was a risk factor for vascular disease. It still is an independent risk factor – we don't know why, but if you give folic acid, you lower homocysteine and then perhaps vascular disease. But what about vitamin B_{12} in countries on very low B_{12} containing diets e.g. in India? The US fortifies flour with folate but Europe generally does not. Perhaps evolutionary biology can help us there?

Doug Wallace: I agree with Philip about the issues but in both Europe and the US we have an increasingly diverse population and we already know that African Americans and native Americans have very different outcomes to the diet and environmental exposures than we see in Europeans. So maybe that information – which is available in the literature – could also be helpful in looking at different populations.

Philip James*:* The Asian immigrants in the UK were also clearly shown years ago to be more susceptible to diabetes even when living in a UK environment (76).

Anne Molloy. If you take fortification with folic acid in the US, the three groups that are being looked at are the Hispanic population that have a high incidence of NTDs and lower folate status than the Caucasians. The Black Americans have the lowest folate status. Despite this the Black Americans have the lowest incidence of NTDs, so clearly here is a genetic background affecting a risk that is polygenic.

David Rosenblatt: I was taught by Clarke Fraser, Canada's first medical geneticist, who popularised the concept of multifactorial disease. I will be audacious enough to use the model of the potato famine in Ireland and the migration of the Irish to North America. The incidence of neural tube defects was highest in Ireland and decreased when they came to North America. But they were still higher than the other North American populations that was there at the time. So you are again looking at an interaction of some sort of genetic pre-disposition with the environment in which the people are placed.

Philip James*:* So despite Patrick's point about the remarkable differences in individual responsiveness to meticulously controlled dietary change WHO at the moment is intent on not trying to specify that the human populations are different because that has all sorts of political and cultural implications. And at the moment the WHO views are reinforced by the growth and development studies in six populations throughout the world where they took well-fed women from 6 populations from very different ethnic backgrounds and showed these women under favourable circumstances produced babies that were almost identical in size with a very small standard deviation in the observations made (77). They had,

however, ensured that they were fully breast feed for 6 months before being weaned in the approved WHO manner with full immunisation etc. They found with the children followed up to the age of 5 years there was a most remarkable consistency in growth. So given this almost identically growth across the populations and the variance within any one population being astonishingly small everybody is then saying if you control the environment then the human race is essentially the same!

Mark Thomas: So it is clear that there are genetic variations that underlie disease susceptibility and this is geographically structured – that is very clear. I suggest, when you are dealing with people like the World Health Organization and so on, stop using categories like Caucasian, or Black or whatever – these are not meaningful biological categories. You can talk of bio-geographical ancestry which is fine and by avoiding these categories you will stop upsetting so many people!.

Doug Wallace: I think one of the complications we have had in relation to finding genetic variation as it relates to predisposition to disease is that a very large resource has been expended on genomic analysis looking at associations for complex disease. But they have explained a modest amount of the variance. So that has resulted in, I think, a loss of confidence in the idea that there might be genetic variation that is relevant. But I think all the points that have been made over the last 2 days point out that maybe the problem is that we have been too focused on one of the variables. So our example of mitochondrial DNA variation – this is a lot cleaner than nuclear variation – it is only a single locus but explains a lot more of the variance than the nuclear variation. The epigenome is something that we can't quantify. So I think that you need to tell WHO they may be using current data that is not generalizable to the overall situation.

Sarah Tishkoff: I have had a lot of discussions at NIH and elsewhere about population differences. There are real problems when we refer to a biological classification of race but if you substitute the issue of ancestry then we need to think about adequate analyses. One thing that NIH has recognised is that minority populations are gravely under-represented in genomics research.

One of the problems is that undertaking research in say African American communities induces a lot of hesitation in those communities because there have been abuses in the past. So long-term relationships have to be built and trust established. But then when applying for funding we find in genome-wide association studies that they always demand large numbers in each population to attain statistical power. You will never have the same study sizes as those conducted in Iceland or in some of these giant cohorts analyses in Europe. So what we are trying to push for is for NIH to pass some sort of actual formal policy on the approach needed. Just to give you an idea of how sensitive this issue is - I have worked with an African-American social scientist who gets very upset and considers it an offensive statement if you say in the African American community there is a higher risk for hypertension or diabetes. She will really get upset and ask whether you are telling me that I am genetically inferior?. But then I point out to her that I can't think of a single population where there isn't a particular disease that is at higher prevalence in that population due to local adaptation and that particular environment but she then immediately questions as to whether this simply reflects the disadvantageous circumstances of the African American communities. And she is absolutely right but both genetic and environmental factors are probably involved.

Anne Molloy: If the folate trials for NTD had been conducted in Africa, we would all be saying folic acid has nothing to do with NTDs whereas it prevents about 72% of NTDs in Caucasians and has a very large effect indeed in Hispanics. So there is a genetic factor in African Americans.

David Rosenblatt: There is a lot of work being done in Canada on First Nation populations and when Rod McInnes was President of the American Society of Human Genetics he actually spent a lot of time on this at one of the meetings in terms of ensuring the community was involved. So I was actually wondering how you handled consent and

involvement of the community in Africa when you were actually doing those studies and then how does the NIH respond to that type of analysis?

Sarah Tishkoff: We have to be exceptionally careful because at that time there was a lot of what people called 'Helicopter genetics'. People were coming in, taking samples, and then just leaving. To do this research properly, we knew that we had to do this in an ethical manner so that this would end up being a long-term study. I had to go through ethical reviews in every country and often with multiple reviews – some of which had more to do with political aspects. The average time to get a permit is on average 5 years!

David Rosenblatt: Were the people in the community involved in framing the project?

Sarah Tishkoff: We worked together with our African collaborators and they would play a role but this did not happen with the Hadza or Pygmy studies.

David Rosenblatt: Laura Arbour, a medical geneticist in British Columbia who worked with the First Nation community showed it really has to be a community project that they are developing with your advice and support and input (78). They have to frame it in terms of their priorities.

Sarah Tishkoff: We always undertake a community discussion, and you want ideally to get community consent, not just individual consent. So that is certainly a goal and we have to explain why we are interested in this kind of research and whether there are benefits or not. You have to be to tell them if they are not going to directly benefit. Actually people are intelligent and they get it: so we have worked with communities where if you talk about inheritance – in Africa there is already a belief their ancestors are in their blood and if you explain it in terms of family resemblance and things like that, people absolutely get it. The other thing is that you actually have to go back to the communities year after year and we return results to them. When I have done that in one community in a very rural area this guy had a pamphlet about science and there was a double helix and he asked me is that what you are studying? He then asked whether we could trace our African Americans in the US back to Africa? Not everybody is going to get it to that level but we try to explain it in that way.

Bill Leonard: We found it very productive in our research in both Siberia and Latin America and in South America to draw in local community members as participants in the research itself and they can be vitally important in helping to explain what we are doing but also underscoring to individual family members and participants the value of maternal/child health, and the various health-related parameters that we are interested in. So building local capacity is I think critically important to doing this kind of research in an international context.

Sarah Tishkoff: I completely agree with you. We did find it very helpful in each of the communities when we involved someone – sometimes a local doctor or nurse - it makes a huge difference. The one thing we will ask every community is what their major health concerns are. I can't promise them that we are going to right each and every one of those concerns, but it is something that informs our approach.

Philip James*:* So we have actually had a fascinating discussion over 2 days and some of us have learned about the astonishing developments in our understanding of the way in which mankind has evolved over the millennia. We have recognised that food is a fundamental interacting lock-in and it is of enormous importance. Then we learned that we have been taking a rather primitive approach by just looking at indices such as genomic scanning alone. We are beginning to realise that we have to be much more sophisticated if we are to understand the pressures that particular groups adapted to developmentally. We are therefore now realising that we are going to have to confront the issues of the diversity of mankind and how to cope with these issues in an appropriate way and ensure our analyses are based on good evidence. So I think this Marabou meeting has been astonishing in allowing us to progress so easily from some of the most fundamental genetics issues through to the major dilemmas in public health. So I thank you for being here and for all your input.

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