assumed by Kimura. Alternatively, if $m < ur$, N_e is the appropriate population size, but in this case hybrids between populations would be heterozygous at almost all loci, and would be polymorphic for different alleles at any given locus. There is evidence (Stone *et al.*, 1968) that for Drosophila on pacific islands, this is not the case. In fact, if $m < ur$ for a period long enough to affect the equilibrium described by equation (1), we should probably describe the different populations as separate species.

It seems therefore that the agreement with equation (1) obtained by Kimura for Drosophila is spurious, and that if neutral mutation is as common as he supposes then a far larger proportion of loci should be heterozygous in wild populations than is in fact the case. Unfortunately, this discrepancy is insufficient to disprove the neutral mutation hypothesis, since it may be that Kimura has seriously overestimated the neutral mutation rate. The difficulty arises because in Drosophila we cannot estimate u from the rate of gene substitution, as might be possible in the case of mammals, since no proteins have been sequenced.

Equation (1) should however provide a way of testing the neutral mutation hypothesis. For example, comparing different species of mammals, one would expect a far higher degree of genetic polymorphism in, for example, a rodent with a continental distribution than in a large ungulate confined to a small geographical range.

There is however a serious difficulty in using equation (1) to test the drift hypothesis. This is that it describes an equilibrium which is reached very slowly. The number of generations required to approach the equilibrium. (e.g. after a disturbance due to a change in N_e) is of the same order of magnitude as N_e; hence no large population will be anywhere near its equilibrium. This is particularly serious when considering a species such as our own, which has increased by a factor of perhaps 10^4 in the last 400 generations. Nevertheless, there may be a way of testing the neutral mutation theory from data on the frequencies of variant proteins in man.

5. Gene frequency distributions in man

The approach to an equilibrium between mutation and elimination is exceedingly slow in a large population. Hence if we want to test the neutral mutation theory by comparing observed and theoretical gene frequency distributions, we need to work out what the gene frequency will be in a population changing in size. It turns out that this may be possible for a population such as our own which has increased by several orders of magnitude in the recent past.

Every gene at a particular locus in the present population is a copy, without mutation, of a gene which arose by mutation some specific number n generations in the past. Let F_n be the probability that a gene, chosen at

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random from the present population, arose exactly n generations ago. Let N_0 and N_n be the population size now, and n generations in the past respectively.

The number of new selectively neutral mutations arising exactly n generations ago was $2\,N_n u$. If, as we must on the drift hypothesis, we ignore selectively advantageous mutation, the expected number of copies now of every gene (including new mutations) present n generations ago is $N_0\,(1 - P_{n-1})/N_n$, where P_{n-1} is the fraction of genes now which are copies of genes arising by mutation during the last $(n - 1)$ generations. Hence if $P_{n-1} \ll 1$, we have $F_n = 2\,N_n u \times N_0/N_n \div 2\,N_0 = u$ *.

Thus the neutral mutation theory leads to the very simple conclusion that, provided $nu \ll 1$, the fraction of genes now present which arose by mutation during the period from r to n generations ago is $(n - r)u$. This can form the basis for a frequency distribution of variant alleles in the present population.

The derivation of frequency distributions is now being undertaken. However, it already seems likely that the frequency of rare haemoglobins in man will not agree well with the frequencies predicted from the neutral mutation theory. Considering the observational data first, Lehmann (quoted in Harris, 1970) in a sample of 10971 individuals from Western Europe found 10 rare electrophoretically separable haemoglobin variants, 3 in the α and 7 in the β chain. Of the β chain variants, 3 are common in other parts of the world and thought to be maintained by heterozygous advantage. Of the remaining 7 variants, 4 occurred once and 3 twice. Hence all had frequencies less than 10^{-4}. Together, these very rare variants contribute a fraction of approximately 2.5×10^{-4} of the genes at each of the α and β loci.

An idea of the distribution to be expected on the neutral mutation theory can be obtained as follows. Let the probability that a new mutation arising n generations ago is still represented in the population be P_n. For all mutations occurring n generations ago the expected number of copies now is N_0/N_n, and hence for each surviving mutation the expected number of copies is $N_0/N_n P_n$, and hence its expected frequency now is $1/N_n P_n$. For $n > 400$ the human population was probably small - perhaps of the order of 10^5. P_n for $n > 400$ will be of the order of 10^{-2} or less. Hence alleles arising by mutation 400 generations or more in the past will, if still present, have frequencies of 10^{-3} or greater. No such alleles were found in Lehmann's samples (if we discount the alleles thought to be maintained by heterozygous advantage), although if the period from 0 to 400 generations ago has provided a fraction of 2.5×10^{-4} of existing genes, we would expect that the period from 400 to 4000 generations should provide 2.5×10^{-3} of existing genes, or perhaps one or two alleles with frequencies of 10^{-3} at each of the α and β loci. We would also expect some alleles with still higher frequencies originating still further in the past.

* The precise expression for F_n, when P_{n-1} is not very small, is $F_n = u\,e^{-un}$.

The objection might be raised that it is inappropriate to consider electrophoretically separable variants as typical of selectively neutral variants. But in the evolutionary divergence of the α and β chains, there have been 60 substitutions which do not involve a charge change and 24 which do, corresponding roughly to the ratio to be expected if the two classes are equally likely to be selectively neutral.

The simplest explanation of the discrepancy is that selectively neutral mutations do not occur. The very rare variants are slightly disadvantageous, but have not yet had time to be eliminated. Variants arising more than 400 generations ago have been eliminated.

There is however one other possible explanation, consistent with retaining the view that most gene substitutions in evolution are selectively neutral. Suppose that our species has in the comparatively recent past (say 5000 generations, or 100,000 years) gone through a " bottleneck" of very small numbers, leading to genetic homozygosity at many loci, including haemoglobin. On this view, the absence of variant haemoglobins with frequencies of 10^{-3} or greater is explained by inbreeding *. This view would be consistent with the existence of other loci in man in which two or a small number of selectively neutral alleles were common, since the degree of inbreeding might have been sufficient to produce homozygosity at some but not all loci. But it would be inconsistent with the presence of loci at which an appreciable number of alleles have frequencies of 10^{-3} or more.

If there has been such a bottleneck, it should be possible to estimate approximately how small the population must have been. The bottleneck must have occurred in the last 100,000 years, since if it had occurred earlier we would expect to detect more variants which have arisen since that time.

More work, both in collecting data on protein variants and in deriving the theoretical distributions, is needed before drawing any firm conclusions. Perhaps the most interesting possibility is this. If we are obliged by other evidence to accept the neutral mutation theory, then we may be able to obtain an idea of the size of populations ancestral to our own from a study of protein variation in existing populations.

* The idea that close inbreeding would lead to an absence of variant proteins is not inconsistent with the equation $F_n = u\, e^{-un}$. In this equation, F_n is the *mean* fraction of genes in the present population which originated n generations ago. If the human population was closely inbred in the past, the variance of F_n would be very large. Thus imagine a large number of populations each having the same past history of numbers as our own. Then if this past history involved recent close inbreeding, F_n gives (approximately) the probability that a particular population is homozygous for a gene originating n generations ago. But if there has been no recent inbreeding, F_n will approximate to the fraction of each population consisting of such genes.

References

J.B.S. Haldane, *J. Genet.*, **55**, 511 (1957).

H. Harris, *Proc. Roy. Soc. B*, **164**, 298 (1966).

H. Harris, *The principles of human biochemical genetics.* North-Holland Publishing Co. (1970).

M. Kimura, *Nature, Lond.*, **217**, 624 (1968).

M. Kimura, *Proc. Natl. Acad. Sci. U. S.*, **63**, 1181.

M. Kimura & J.F. Crow, *Genetics*, **49**, 725 (1964).

J.L. King, *Genetics*, **55**, 483 (1967).

J.L. King & T.H. Jukes, *Science*, **164**, 788 (1969).

R.C. Lewontin & J.L. Hubby, *Genetics*, **54**, 595 (1966).

J. Maynard Smith, *Nature, Lond.*, **219**, 1114 (1968).

J. Maynard Smith, *Amer. Nat.*, **104**, 231 (1970).

R.D. Milkman, *Genetics*, **55**, 493 (1967).

W.S. Stone, M.F. Wheeler, F.M. Johnson & K. Kojima, *Proc. Natl. Acad. Sci. U.S.*, **59**, 102 (1968).

J.A. Sved, *Amer. Nat.*, **102**, 283 (1968).

J.A. Sved, T.E. Read & W.F. Bodmer, *Genetics*, **55**, 469 (1967).

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DISCUSSIONS

C. LEVINTHAL : What is the relationship between Lewontin's experiments and the lack of variation you discuss ?

J. MAYNARD SMITH : Lewontin was one of the first population geneticists to exploit protein variation. He is one of the people responsible for my dogmatic statement that populations are a great deal more variable than we had thought. When he first found this, he thought it must be explained by neutral mutation, because the extent of it is too great to be accounted for by natural selection. He said this because he, as we all did at that time, accepted Haldane's cost of selection argument. However I think Lewontin would now accept that this protein variation could be explained either way. Since that time, Lewontin and others have studied protein variation in flies from different local populations of the same species — for example on different islands. If you have a population divided up into small groups and it turns out that each group has the same variant, then this is very difficult to explain on a random basis. It suggests that each group must be stable, otherwise they wouldn't be the same, they would drift apart. A lot of work of this kind is going on at that moment. It's difficult to interpret precisely because of the difficulty that the equilibrium is approached very slowly. Unless you know quite a lot about the past numbers of the animal you are looking at, it's difficult to interpret the data. I have a feeling that our own species, for which we have more idea about past population numbers than for the fruit fly, may be a better bet than fruit flies because of this particular problem.

H. FRÖHLICH : Is there anything known about the physical processes that lead to a mutation ?

D. GLASER : I have been asked to answer this, which I will do by expressing, the prevailing prejudice that, except for occasional hot spots, the probability of a base change is the same everywhere in the chromosome. Whether a base change is reflected in an amino-acid change which can have a phenotypic effect, depends on the considerations that Pr. Margoliash outlined for us. Since leucine is represented by four possible base triplets and methionine by only one, a particular protein rich in leucine, can tolerate more base changes without suffering a change in the amino-acid leucine, then if it is rich in

methionine. In general, I think, the prevailing prejudice is that the probability of a base change is uniform along the DNA, but doesn't produce uniform consequences in the amino-acids. And then it's a very open question which Pr. Margoliash discussed at length, whether amino acid changes produce phenotypic changes. But maybe we can get a professional answer.

D. GLASER : I have a question : is it really necessary that we decide between the two competing theories. Isn't it more likely that there is a range of proteins, some of which can sustain large numbers of neutral mutations, and others of which, because of the sensitivity of their structure to their sequence or because of their critical importance to the organism, cannot tolerate the slightest change in their structure.

E. MARGOLIASH : It is important to point out something different. Lewontin's experiments are also based on electrophoresis of *Drosophila* extracts. Therefore, the estimate of heterogeneity obtained is a minimal one, since not all changes of structure necessarily produce an electrophoretic variation. We may in fact be dealing with much more than he actually observed.

J. MAYNARD SMITH : I think you are right that we don't have to take a black or white choice here. However I should perhaps explain that in the history of population genetics almost religious feelings were aroused by the problem of whether anything could be selectively neutral. There are men, you know, who will go to the length of saying that nothing can possibly be selectively neutral. So for such people, once you admit that any mutation is selectively neutral, you have really sold out to the devil. Now I don't take this view. I think it perfectly possible to say that some proteins, say fibrinopeptide, can vary a lot without mattering, others are very tightly constrained. But in Dr. Margoliash's study of cytochrome c., it will probably turn out that one of two things is true. Either the great majority of the variations are selectively neutral, or the great majority have a selective interpretation which for the moment we cannot see. I'd be surprised if it turned out that 50 % was of one class, and 50 % was of the other. But that's just hunch.

J. BRONOWSKI : I want to ask a question about this last argument which is particularly interesting because it is quite new. Are not the calculations going to be seriously affected by whatever assumptions you make as to when perfect mixing began to take place ? In other words, how many highly homogenous human populations which were separated 500 generations ago have entered the stream that you are measuring now ? And, of course, the analysis also makes assumptions about selective mating which run all through the argument.

J. MAYNARD SMITH : This is the real difficulty. However it may not be as severe a difficulty as at first one might think. I have been looking at the problem of how much interchange of genes there must be between populations in order, for the purpose of these calculations, for them all to be effectively one population. A degree of migration of the order of the mutation rate is all you need. So, unless one supposes total isolation, then I think probably the distortion won't be too big.

D. GLASER : I would like to make a comment about this calculation you say is in progress, and express pessimism that it will be useful. That process is not unlike the problem in bacterial genetics of counting the number of mutants in a population and trying to determine the mutation rate, as was done in the interpretation of the Luria-Delbrück experiment. The mathematics for that problem after many years of approximations has finally been worked out exactly by Benoit Mandelbrot and leads to a statistical formula in which enormous fluctuations are found in the final number of mutations. The fluctuations are so large that it is extremely difficult to give any kind of estimate about the mutation rate which one wants to determinate.

J. MAYNARD SMITH : I was worried by this as well. Of course you can calculate the means; it is calculating their variances which is difficult. But there is this difference. If we wanted to know how many mutations we expected to find in the existing human population which originated exactly 500 generations ago, it is fairly easy to estimate the mean. But its variance might be so big that it would mean nothing. This is essentially the problem in the Luria-Delbrück situation. We do not have this problem because I'm not interested in how many mutations originated exactly 500 generations ago. What I'm interested in is how many originated between 500 and say, 5 000 generations ago. Then I think the variances are going to shrink fairly fast. However the variance might remain large if the human population has been through a bottleneck of small numbers of the order of 100.

D. GLASER : In Mandelbrot's analysis, the statistical distributions are of the Pareto type, $P(x) \propto x^{-\alpha}$, $0 < \alpha \leqslant 2$, and do not possess mean values, but only percentiles.

G.M. EDELMAN : In your last example, wouldn't recombination play a big role if there were selection for heterozygosis in the population. I seem to remember that Wills and Lewontin have talked about this possibility. Wouldn't it tend to increase the amount of variation and possibly account for these results ?

J. MAYNARD SMITH : Yes indeed, but only if selection is involved, not if the mutations are neutral. The beauty of the neutral mutation theory is the mathe-

matics are much easier, so that we can work out the consequences of the neutral theory with adequate precision. We can then compare the predictions of the neutral theory with observation, and if that doesn't fit, then we know there's some selection, and that is the basic point we want to decide. If there is selection we shall have to start worrying about the point you raised about recombination, but on the whole population genetic calculations become dreadfully difficult as soon as you remember that genes are on chromosomes and are linked.

P.O. LÖWDIN : I will try to comment on Pr. Frölich's question concerning the physical reasons for mutations. The problem is not to give a series of possible mutation mechanisms, but to explain why mutations are so exceedingly rare in each one of them. If you look at the Watson-Crick model of DNA and the genetic code, it may be a good idea to start out with doubts and try to prove that the model is wrong or physically impossible — which is the starting point we chose in Uppsala in 1962 in our quantum-mechanical studies. The genetic code consists of a pattern of protons and electron-pairs forming hydrogen bonds, and, in each bond, the proton has two classical equilibrium positions corresponding to different information storage :

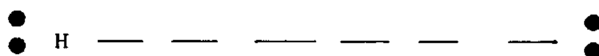

Fig. A

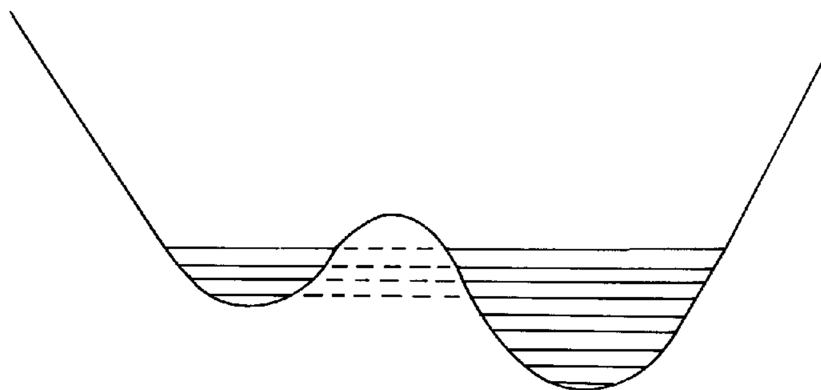

Fig. B

In quantum mechanics, the wave packet associated with the proton may " tunnel " between the two classical equilibrium positions, and the occupation of the tunneling levels depends on the temperature. At body temperature (310 °K), the tunnel effect corresponds to an " error " of 10^{-10} and 10^{-11} for the GC- and AT-base pairs, respectively, and these errors are associated with spontaneous mutation probabilities per base pair and generation. The

fact that these figures are so small indicate an essential stability of the genetic code in the Watson-Crick model; the corresponding experimental figures are estimated to lie between 10^{-8} and 10^{-12}. It is interesting that the AT-pair seems to be more stable than the GC-pair. If the nucleic acid is exposed to weak ultraviolet radiation, there are electron transfers in the base pair which may enhance the probability for a proton transfer by a factor 10^{6}.

References :

Per-Olov Löwdin, " Quantum Genetics " (*International Science and Technology*, May 1963).

Per-Olov Löwdin, " Quantum Genetics and the Aperiodic Solid. Some aspects on the Biological Problems of Heredity, Mutations, Aging, and Tumors in View of the Quantum Theory of the DNA Molecule " (*Adv. Quant. Chem.*, **2**, 213 (1965)).

Per-Olov Löwdin, " Some Properties of the Hydrogen Bonds in Biochemistry with Particular Reference to the Stability of the Genetic Code " (*Pontificiae Academiae Scientiarum Scripta Varia* 31, « Semaine d'Etude sur les forces moléculaires » (1966)).

S. Bennett : With reference to the question by Professor Bronowski, I wish to express my lack of understanding at the choice of 500 generations as fitting some significant point in human evolution. If I judge this correctly, this would be about the end of the Pleistocene and somewhere in the neighborhood of 15000 years ago. At that time there were many isolated human populations in many parts of North America and the Old World. To postulate some single genetic pool, or even a small bottleneck of some kind, strikes me as unreasonable. For this reason, I don't understand the merits of choosing this kind of a basis for the argument you present. It seems to me a much older, much longer series of generations would be necessary, if one is to draw on our present knowledge of human evolution.

J. Maynard Smith : The choice is not based on our knowledge of human evolution in this case. The point is that comparatively recent mutations, let us say those occuring in the last 200 or 300 generations, would have very low frequencies in the present population. So that the known observation that there are a number of variants of haemoglobin with a very low frequency of 10^{-4} or less is consistant with the idea that these have originated in the last 300 generations. But if a mutation originated more than about 500 generations ago, and if such a mutation is still present in the population now, then it would probably have a frequency of more than 10^{-4}, and such variants have not been found.

K. Mendelssohn : May I just ask the chairman one thing : is there really a difference between the thermal mutation rate and mistakes in arrangement.

Are mistakes in arrangement necessarily part of the thermal or statistical variation under kT ?

D. GLASER : I'm sure that if you did a calculation you would probably identify several terms that contribute to the total rate. One term would be thermal dislocations in complete double helix structures; another would be thermal damage to a temporarily open singlestranded structure; a third one would be thermal disturbances in the fitting of the polymerase to the precursor and single-strand structure that it was working on; and probably another one would be a simply a quantum mechanical calculation of barrier penetration probabilites independent of kT. What I had in mind was the last one, when I spoke of quantum mechanical effects which were not thermal. I'm sure all these things would come together in determining the temperature dependence of mutation rates, for instance, but I imagine you have to estimate them separatly.

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PHYSICAL AND CHEMICAL PROCESSES LEADING TO A MUTATION

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Mutation and selection are generally regarded as the main driving force of evolution. We have now a good classification of mutations : nonsense, missense, frame shift, deletions and chromosome aberrations are the main kinds, covering most of the events. A more complicated problem is how mutations are generated, what is the mechanism of the premutational act, how they become fixed and replicated in the progeny ? I shall concentrate on two different types of mutations — the spontaneous and induced ones. Both were studied in our laboratory using bacteria as a genetic model. What are spontaneous mutations ? Their probability is on the order of 10^{-7} - 10^{-9} for a specific gene per generation of cells. If we take for the number of acting genes 10^{2} (the overall number of genes per bacterium is on the order of 10^{3}, but only a part of them is derepressed), so the number of mutations in any active gene would be 10^{-5} - 10^{-7} per generation. If we take 10^{3} nucleotide pairs as an average dimension of a cistron it gives us the probability of replication mistake on the order of 10^{-8} - 10^{-10} per generation. We will take 10^{-9} as a mean. If this is an intrinsic probability of erroneous replication by DNA-polymerase during DNA synthesis we must consider a rate constant of the reaction of noncomplementary DNA replication. For bacteria the rate constant of regular DNA replication is on the order of 10^{4} nucleotide pairs/second. Hence, for the rate constant of erroneous noncomplementary replication we obtain $10^{-9} \times 10^{4}$ nucleotide per/sec. = 1 nucleotide per/day. We see that if we could stop the reaction of regular DNA replication but keep the cell alive and the DNA-polymerase active, we would obtain one wrong nucletide addition per day. This would be a premutational event. If the cell would grow afterwards and replicate its DNA in a normal way a mutant would result for every cell in an entire population. This experiment was really performed recently. We took thymine deficient cells of Bac. subtilis and E. coli and cultivated them in a medium with a very low concentration of thymine (0.2 - 0.3 μg/ml). It is a usual practice that bacterial cells die out in such conditions during 2 - 3 hours. The phenomenon of thymineless death was much studied last years and was regarded as a very general one. But we found that on a solid medium i.e. agar plates with a minimal medium supplemented with a very small amount

of thymine or devoid of thymine, thymineless death is practically absent. Cells can be washed off after 50 - 80 hours of thymine starvation and the cell count is constant. When a small thymine concentration is given, the cells even grow and divide. The surface of agar, where at first 10^4 - 10^5 cells were plated (on a Petri dish) becomes covered by microclones visible in the microscope. The cells are washed off afterwards and cultivated in a medium with 20 μg/ml of thymine and then tried for mutants. In conditions of thymine starvation an overall mutagenesis in the whole cell population develops. It starts after 20 hours, when the growth of the cell population comes to a stop. During 2 - 3 additional days the per cent of auxotrophic mutants increases and comes to 70 - 80 % of the whole cell population. Practically most of the cells are mutants and many of them multiple mutants. It is easy to observe the auxotrophic cells with deficiencies for aminoacids, nucleotides, vitamines. They do not grow on minimal agar or grow at a small rate (if the mutants are leaky). We identified some 10 - 12 of the mutants in different loci. We studied also revertants to prototrophy in tryp locus and drug resistant mutants (especially streptomycine resistant).

The frequency of any particular mutation was some 10^3 times higher in our conditions than in the control. (For instance the number of streptomycine resistant cells increased without selection till 10^{-2} % of the cell population, with less than 10^{-5} % in the control, growing without thymine starvation). Of course some of the mutations are lethal and this gives a small decrease of the cell population (some 30 - 40 %). It is obvious that we deal here with a phenomenon of complete mutagenesis in an entire cell population, mainly without death of the cells involved. We think that the only possible explanation is erroneous DNA replication. The scale of time for the occurence of mutations by replication is just what we estimated earlier. The frequency of erroneous replications is a function not only of DNA structure and the properties of nucleotides. It depends also on the enzyme specificity. This factor was studied by Yanofsky and others and special strains with mutated DNA-polymerase revealed increased probabilities of replication mistakes. This is obviously the explanation of special mutator genes- i.e. genetic loci, which imply to the organism and increased mutability and genetic instability.

Now we turn our attention to induced mutations, generated by some chemical substances or by UV-irradiation. We studied them by means of in vitro reaction with isolated and purified DNA with subsequent biological control of the DNA changes by means of transformation of bacteria (Bac. subtilis particularly). Of course we do not take here into consideration the mutations caused by aberrant DNA replication like those considered above. We can mention as example of the latter the increased mutability of bacteria with-5-bromouracil incorporated into their genome instead of thymine, probably because of tautomeric changes in the molecule, and also some specific muta-