THE GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers presented at the HUNDRED AND SEVENTY-SEVENTH MEETING of the Society held on 25th and 26th March 1975 in the UNIVERSITY OF KEELE.

SEQUENCE EVOLUTION IN DNA

P. M. B. WALKER

MRC Mammalian Genome Unit, Department of Zoology, University of Edinburgh

Evidence is accumulating that major fractions of the nuclear DNA evolved by methods very different from that which specifies proteins. The clearest example of this kind of DNA are the satellites and spacers, but present methods would not detect highly diverged sequences of satellite origin. There is also at least one clear case of the majority of the DNA being essential for chromosome structure and only a minority needed for protein specification.

THE BASIS OF MAMMALIAN CHROMOSOME BANDING

M. BOBROW

Genetics Laboratory, Department of Biochemistry, South Parks Road, Oxford

About four years ago the introduction of various chromosome banding techniques revealed that mammalian chromosomes have a varying structure at different points along their length. Several hypotheses have been put forward attributing this variation to differences in DNA base composition, intermediately repetitious DNA, differences in protein distribution, and differences in chromatin condensation. There is persuasive evidence that the banding patterns represent differences in degree of chromatin condensation, presumably correlated with either qualitative or quantitative differences in the distribution of chromosomal proteins. There is circumstantial evidence from a large number of sources, that the chromosome bands may also contain relatively A-T rich DNA.

EVOLUTION OF HETEROCHROMATIN IN PRIMATES

K. W. JONES

Institute of Animal Genetics, West Mains Road, Edinburgh

Highly repetitive simple sequence DNA, commonly referred to as satellite DNA, is concentrated in the constitutive heterochromatin of most of the eukaryotic species so far examined. This fact was established largely by the use of molecular hybridisation and constitutes a specific, if partial, chemical characterisation of this type of chromatin. The facility whereby such chromosomal regions may be characterised, in terms of the DNA base sequences present, forms the basis of an investigation of the chromosomal distribution and evolution of particular heterochromatic structures in the higher primates.

In many species, satellite DNA is characteristic and exclusive, attesting its rapid evolution. In the higher primates, however, there has been conservation of certain satellite DNA sequences of sufficient degree to permit molecular hybridisation to be used as a means of identifying the presence of the related satellite DNA in all of the existing species including man. This confirms the existence of a common ancestral primate as the source of the related DNA sequences.

The chromosomal distribution of the related satellite DNA has been determined by *in situ* hybridisation, and the DNA has itself been purified from certain species for comparative study by physical and other means. Such studies permit some tentative conclusions con-

cerning the evolutionary inter-relationships of the higher primate species, and provide indications of the manner in which satellite DNA has evolved in this group, in comparison with more complex DNA.

The higher primates also exhibit species-restricted satellite DNA's suggesting origins for such DNA subsequent to species divergences. Study of these may provide evidence concerning the relative times of species divergence, and hopefully illuminate the obscure question of the general significance of satellite DNA.

VARIATIONS AND FUNCTIONS OF CENTROMERIC HETEROCHROMATIN

J. FOREJT

Institute of Experimental Biology and Genetics, Czechoslovak Academy of Sciences, Prague

Polymorphism of centromeric heterochromatin (*Hc*), defined by C-band technique, was described in mice of inbred strains and natural populations (Forejt, *Folia biol. (Praha), 18,* 213, 1972; *Chromosoma, 43,* 187, 1973). Further study of wild mice captured at different localities confirmed 'heterozygosity' of about 20% of chromosome pairs for distinct *Hc* variants. Markedly reduced *Hc* regions were predominantly found in longer chromosomes of wild mice.

The possible evolutionary significance of *Hc* polymorphism which would consist in suppressing crossing-over in paracentromeric chromosomal regions was experimentally tested; the frequency of paracentromeric chiasmata in two inbred strains (differing in *Hc* patterns) and in their F_1 hybrids was compared. The expected decrease in paracentromeric chiasma frequency was not confirmed for F_1 animals.

A particular case of *Hc* affinity was studied in meiosis of males heterozygous for reciprocal translocation T(14;15)6Ca. 10-30% of primary spermatocytes (diakinesis, MI) revealed tight attachment between *Hc* regions of chromosomes X and 15. The genetically controlled and age dependent variations in frequency of the tight attachment were observed. A possible relationship of the observed X-autosome *Hc* attachment to impairment of spermatogenesis of T6/+ males is postulated.

OBSERVATIONS ON THE REPEATED-SEQUENCE DNA OF GENOTYPES WITHIN THE TRITICINAE HAVING CONTRASTING DEGREES OF MEIOTIC CHROMOSOME PAIRING

G. A. DOVER

Department of Genetics, University of Cambridge

The "A" and "B" chromosomes of two species of *Aegilops* have marked effect on meiotic chromosome pairing in F-1 hybrids of wheat × *Aegilops* spp. In particular, the "B" chromosomes reduce the high levels of homoeologous (multivalent) pairing normally associated with the absence of wheat chromosome no. 5B. (Dover and Riley, *Nature, 240,* 159-161, 1972; Dover, *Proc. 4th Int. Wheat Gen. Sym. 1973*).

Computer differentiation of mean melting curves of DNA, extracted from single plants of *Aegilops* with varying numbers of "B" chromosomes, has revealed a small A-T rich DNA fraction associated with "B" carrying individuals. This difference is substantiated by small shifts in Tm and buoyant densities of "B" carrying individuals. However 95% of the "B" chromosome DNA is similar to "A" chromosome DNA of wheat in profiles of differentiated melting curves. There are no significant differences, at "Cot" values of 10^{-2} (highly repetitive DNA) or 100 (intermediate repetitive DNA), in reassociation and % heteroduplex formation with wheat DNA, between the DNA of individuals carrying high and low numbers of "B" chromosomes. Thermal elution profiles of homo- and heteroduplexes underline a high level of homology between the "A" and "B" chromosome DNA of wheat and *Aegilops*.

These results do not support the suggestion that highly repetitive satellite DNA might play a role in meiotic chromosome pairing.

HUMAN GENE MAPPING USING CELLS WITH AN X/AUTOSOME TRANSLOCATION

E. SOLOMON, P. N. GOODFELLOW and M. BOBROW
Genetics Laboratory, Department of Biochemistry, University of Oxford

Interspecific somatic cell hybrids in which one parent cell is human and the other mouse are known to lose human chromosomes, while retaining those of the mouse. Assignment of human genes to particular chromosomes can therefore be made by correlating the presence or absence of specific markers with the presence or absence of individual chromosomes. The fact that one can select for, and against, the presence of the X chromosome in such hybrids greatly facilitates the mapping of X-linked genes.

We have used human fibroblasts with a translocation between the X and chromosome 15 to make human-mouse hybrids. While there is normally no selection possible for chromosome 15, its translocation to the X in these cells has permitted us to generate hybrid clones with and without the 15. Karyotypic and biochemical data on these hybrids support the assignment of several human enzyme markers, and beta-2 microglobulin, to chromosome 15.

GENETIC COMPLEMENTATION ANALYSIS IN (SINGLE) HYBRID CELLS AFTER FUSION OF ENZYME DEFICIENT FIBROBLASTS FROM PATIENTS WITH DIFFERENT VARIANTS OF "THE SAME" METABOLIC DISEASE

H. GALJAARD
Department of Cell Biology and Genetics, Erasmus University, Rotterdam

For an increasing number of hereditary metabolic diseases the primary enzymic defect can be demonstrated in cultured fibroblasts. Within a group of metabolic diseases a "similar" enzyme deficiency may result in different pathological and clinical manifestations. To investigate the genetic background of this heterogeneity somatic cell hybridization has been performed on fibroblasts from patients with the same enzyme defect but different clinical symptoms. The activity of the (iso) enzymes concerned was determined both in cell homogenates and in single binuclear hybrid cells using microchemical techniques. After fusion of different combinations of β-galactosidase deficient cells (derived from different variants of GM 1 gangliosidosis) and of N-acetyl-β-D-hexosaminidase deficient cells (from GM 2 gangliosidosis) genetic complementation could be demonstrated. Assays in single hybrid cells revealed that in some instances the enzyme activity was restored to control values within 2 days after fusion. Inhibition of protein synthesis was found to inhibit such a restoration of enzyme activity. Some other examples of application of single cell assays are illustrated in the field of metabolic interaction between enzyme deficient and normal cultured cells.

SOME SIMPLE MODELS OF CONTINUOUS VARIATION WITH FEW GENES

J. M. THODAY and J. N. THOMPSON, Jnr.
Department of Genetics, University of Cambridge

Finding "continuous" variation in segregating populations, or more strictly variation consistent with normality, is commonly taken to indicate a polygenic situation. This often inhibits those whose interests could be furthered if specific effects of underlying allelic substitutions could be studied.

We therefore present some simulated F2 distributions based on simple genetic models. They show how large samples must be to demonstrate departures from normality. They should serve as a warning against basing any assumption that there are not a few genes of large effect on the "normality" or continuity of the distribution of observed samples alone.

NUCLEOTIDE SEQUENCE ORGANIZATION IN THE WHEAT GENOME

R. B. FLAVELL and D. B. SMITH
Department of Cytogenetics, Plant Breeding Institute, Trumpington, Cambridge

Approximately 80% of the wheat genome consists of repeated nucleotide sequences measured by renaturation kinetics in 0·18 M Na^+ at 60°C. Twenty per cent consists of unique or few copy sequences, approximately 8% of which are sequences 800 to 1,000 base pairs long interspersed between repeated sequences. Five per cent of the DNA reanneals so rapidly that it is likely that it consists of palindrome or reverse repeat sequences. A small fraction (10%) of the repeated sequences consists of related sequences tandemly arranged. However at least 65% of the repeated sequence fraction consists of 500-600 nucleotide long sequences tandemly arranged such that adjacent sequences are unrelated. Furthermore, throughout most (80%) of the repeated sequence fraction, sequences which have diverged relatively little are alternately arranged with sequences which have diverged considerably.

THE RELEVANCE OF EXCISION REPAIR TO UV-SURVIVAL OF YEAST

R. WHEATCROFT, B. S. COX and R. H. HAYNES
Botany School, Oxford, and Biology Department, York University, Downsview, Ontario

Although the biochemistry of many repair processes has been described in some detail and the assumption is generally made that cells owe their ability to survive irradiation to their capacity to repair damaged DNA, few attempts have been made to see whether one can, in fact, account quantitatively for the survival of cells in terms of their repair capacity. In the case of UV irradiated cells, not only the rate of killing but the form of the survival curve (shouldered) may need to be explained by repair capacity, since the principal lethal lesions, pyrimidine dimers, are induced linearly with dose over "biological" dose ranges.

Measurements made of the number of pyrimidine dimers excised from the DNA of yeast following various doses of UV indicate that UV progressively inactivates the excision process. These data suggest both the form of and constants in the "repair" term in a general expression relating survival and repair suggested by Haynes (*Radiation Res. Supp. 6*, 1, 1966), namely

$$-\ln S = \text{number of lesions induced} - \text{number of lesions repaired},$$

S being the surviving fraction.

It is found that using this model, the survival of haploid wild type yeast can be closely predicted from the measured excision data. Various assumptions of biological interest have to be made and these are confirmable by considering the behaviour of various UV-sensitive mutant strains of yeast.

PROTEIN POLYMORPHISM IN NORTHERN POPULATIONS OF FIELD MICE (*Apodemus sylvaticus* L.)

A. ARNASON
Department of Biology, University of Strathclyde, Glasgow

An electrophoretic investigation was carried out on protein variation in different tissues of the long tailed field mouse (*Apodemus sylvaticus*). Physicochemical methods were employed to characterise esterase enzymes and other proteins. The distribution of variant forms from thirteen localities in Iceland, Ireland, Scotland, Norway and Sweden were studied. In some cases it was possible to clarify mode of inheritance by breeding.

Serum pre-albumins, albumin and ceruloplasmin are monomorphic, whilst post-albumin and transferrin may be polymorphic. Red-cell proteins A and B are polymorphic, but Hbs were of one type only. Other tissue proteins were found to be uniform, except for a cathodally migrating protein fraction in striated muscle.

Most esterase fractions were found to be nonspecific carboxyl-esterases. Each tissue has its own esterase pattern, but also shares fractions with other tissues. The physicochemical tests, breeding, ontogenetic and population data suggest a mode of inheritance for many esterase fractions, in which the proposed loci Es-1, Es-2, Es-3, Es-4, Es-7 and Es-8 have each two codominant alleles. Es-6 has one dominant "silent" allele, and one recessive "producing" allele. These loci produce esterase in more than one tissue at the same time. There was a difference between all populations tested, the Iceland populations differing most from the rest. It is suggested that chance "founder effects" are the chief factors for the uneven distribution of the protein markers. This agrees with the work of others (Berry, *J. Zool. Lond.*, *152*, 333-346, 1973). The physicochemical characters of proteins in *Apodemus sylvaticus* are similar to those established for other rodents (Holmes and Masters, *Biochem. biophys. Acta*, *151*, 147-158, 1968).

THE HETEROZYGOTE DETECTION PROBLEM IN MAN

J. H. EDWARDS
Infant Development Unit, Birmingham Maternity Hospital, Birmingham

In principle disorders which produce symptoms in the rarer homozygote, or the hemizygote, and signs in the heterozygote could have their incidence reduced by selective mating, selective childlessness or selective abortion.

In practice there are various difficulties both in the diagnosis of heterozygosis and in the exploitation of the options available.

The diagnostic problem involves special reference to the integration of quantitative and qualitative data (normally one or several laboratory measurements and a pedigree). The connexion between diagnostic discrimination and the efficiency and effectiveness of artificial selection is explored.

A CHINESE HAMSTER CELL VARIANT ABLE TO GROW WITHOUT GLUTAMINE

R. H. WILSON
Department of Genetics, University of Glasgow

Starting from a line of fibroblastic chinese hamster cells (C4B) that required glutamine for growth, a glutamine-independent cell variant (C4B.G) was obtained. The C4B.G cells showed changes in morphology and an increase in chromosome number. The altered phenotype was not due to mycoplasma infection and was stable to reverse selection. Enzyme assays showed no increase in the specific activity of glutamine synthetase. By blocking endogenous glutamine synthesis with methionine sulfoximine, it could be shown that the C4B.G cells were able to utilise exogenous glutamine more effectively, although the precise mechanism of the strain's glutamine independence remains to be demonstrated.

IS THERE GENE ACTION IN THE MALE GERM CELL OF ANIMALS?—A REVIEW

R. A. BEATTY
Institute of Animal Genetics, West Mains Road, Edinburgh

Muller and Settles (1927) wrote ". . . the genes in the spermatozoa are in a dormant state, the nature of the sperm cytoplasm and the sperm regions being determined independent of the specific gene content of the sperm . . .". Subsequent work has supported this dictum. However, there is evidence of DNA-polymerase in spermatocytes and spermatozoa and of RNA synthesis in late meiosis, while at the T-locus of mice and the segregation-distortion locus of Drosophila there appears to be an association between the genetic content and the

fertility of individual spermatozoa. Further, there is a current hypothesis that "inexact" chiasma formation is a very common event preventing the majority of spermatozoa from reaching or penetrating an egg. Muller and Settles left open the possibility that gross abnormalities in the quantity or arrangement of the chromatin might affect sperm phenotype, and there is evidence that diploid spermatozoa do not fertilise eggs.

ONSET OF GENE ACTIVITY IN THE EARLY EMBRYO

R. L. GARDNER
Department of Zoology, South Parks Road, Oxford

Experimental manipulation of the preimplantation mammalian embryo has provided evidence that blastomeres are totipotent at least until the 8-cell stage, and that subsequent differentiation depends on the interactions between naive cells rather than segregation of cytoplasmic determinants present in the egg. Restriction of potency is clear by the 64-cell stage in the mouse, and cells appear to be partitioned into 4 distinct tissue compartments by the approximately 100-cell stage. This leads one to anticipate early onset of genetic activity in the embryo.

Various biochemical and genetic studies argue that this is indeed the case. Synthesis of all major classes of RNA has been detected as early as the 2-cell stage in the mouse. Protein synthesis in early embryos is relatively resistant to actinomycin D and may, therefore, depend in part on translation of RNA molecules made during oogenesis, as seems to be the case in certain non-mammalian species.

Nevertheless, the paternally inherited isozyme of glucose phosphate isomerase can be detected by the 8-cell stage. Furthermore, certain lethal genes lead to death of homozygous embryos at defined stages shortly thereafter. Hence it is clear that parts of the embryonic genome are expressed very early in mammalian development.

POSITION EFFECT VARIEGATION IN THE MOUSE

B. M. CATTANACH
MRC Radiobiology Unit, Harwell

In *Drosophila*, V-type position effect variegation is almost invariably associated with rearrangements in which the wild type of alleles of genes normally located in euchromatin are brought into close association with heterochromatin. The equivalent situation is found in the mouse but here the heterochromatin is provided by the inactive X of the female and, thus, the variegation is most often indistinguishable from that resulting directly from the random inactivation of one or other X. However, the position effect variegation can be observed alone under certain controlled conditions.

Drosophila position effect variegation is thought to derive from an early determinative event by which each cell is subject to a programme of gene suppression, each cell perhaps having a different programme and this, once established, being permanent and inherited clonally. Studies upon the position effect variegation caused by the T(7;X)Ct translocation in the mouse suggest that in this species variegation stems from both early and later events and that the latter represent the reactivation of previously inactive loci. The loci the furthest removed from the break point are likely to be reactivated first, and most frequently. It is proposed that the mechanism in *Drosophila* is basically the same as that in the mouse but less readily recognisable.

DNA MODIFICATION MECHANISMS FOR THE CONTROL OF GENE ACTIVITY DURING DEVELOPMENT

R. HOLLIDAY AND J. E. PUGH
National Institute for Medical Research, Mill Hill, London

The development of complex organisms depends not only on the spatial organization of their cells and tissues, but also on the temporal activity of their genes. There must be mechanisms which control the following developmental features: (1) the segregation of cells with different gene activities; (2) the stability of the determined or differentiated cells; (3) the specific changes which occur in groups of cells at particular times in development, and (4) the differential activity of homologous genetic regions, as in X chromosome inactivation.

We have suggested that all these aspects of development are due to changes in DNA, and that they are carried out by enzymes with specificities similar to the restriction-modification enzymes of bacteria (Holliday and Pugh, *Science*, *187*, 226, 1975). These enzymes would recognise specific base sequences adjacent to structural genes, and the modification of particular bases would control transcription. Modifications could lead either to transient switches or to stable changes in gene activity. Developmental clocks, which activate genes after a specified number of divisions, might be based on successive modification of repeated sequences of DNA. X chromosome inactivation can be explained by an initial switch, together with the sequential modification of specific sites along the chromosome by processive enzymes.

Our model depends on a continual interaction between specific modification enzymes and specific DNA sequences. The combination of developmental clocks and precise segregation mechanisms, which together determine which genes will be activated, provides the essential requirement for an ordered genetic programme for development.

GENETICAL SOCIETY OFFICERS AND COMMITTEE

President	J. M. Thoday
Vice-Presidents	J. H. Edwards, Sylvia D. Lawler, D. A. Hopwood
Treasurer	Mary F. Lyon
Secretaries	M. A. Ferguson-Smith, Ann C. Chandley
Committee	A. W. F. Edwards, C. E. Ford, B. J. Kilbey, D. B. Roberts, S. Baumberg, D. A. Ritchie, Ruth A. Sanger, J. D. Smith, J. A. Beardmore, C. H. Clarke, J. Hill, K. Jones
Membership Secretary	D. A. Smith