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SEX-LIMITED MIMICRY: SEXUAL SELECTION OR GENE DOSAGE, OR BOTH?

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The limitation of mimicry to the female in many butterfly species has been explained by two "rival" hypotheses, the sexual selection theory of Belt (that male patterns are subject to conservative selection) and the gene dosage hypothesis of Stehr (that butterflies produce sex-limited mutations more readily than other insects because the lack of dosage compensation on the sex chromosomes makes it easy for an autosomal gene to "know" whether it is in a male or a female). As often happens with rival hypotheses, both appear to be correct. Belt's hypothesis is consonant with what we know of the relative strengths of sexual selection in male and female butterflies (Turner, *Biol. J. Linn. Soc.*, *10*, 385-432). Although modification of expression is theoretically possible, the genetical evidence points to the genes that have been selected in this way having been female-limited from the start; we have recently confirmed the absence of dosage compensation in the sex chromosomes in butterflies (*Heredity*, *43*, 71-77), so sex limitation probably is readily available. These hypotheses further suggest that female-limited characters should be predominantly controlled from the autosomes, but that male-limited characters should often be X-linked. This too is consistent with what is known in butterflies.

MATING BEHAVIOUR AND SEX CHROMOSOME EVOLUTION IN *SIMULIUM ERYTHROCEPHALUM* (DIPTERA: SIMULIIDAE)

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Two of the chromosome one inversion sequences of *S. erythrocephalum* are partially sex linked, and appear to be correlated with speed of larval development. Sex chromosome linkage is such that the faster karytopes usually occur in males. It is suggested that this is the result of selection. Males which eclose earlier will have potentially more mates, whereas females which spend longer in the larval stage have more ovarioles. Selection of this sort, which acts to improve linkage between the primary sex determining gene and other loci which have alleles exhibiting differential fitness in the two sexes, could result in the accumulation of sex chromosome crossover suppressors, which is a pre-requisite for Y-chromosome degeneration.

REDUCTION OF PSEUDOLINKAGE IN THE T_1/+ TRANSLOCATION HETEROZYGOTE IN *AEDES AEGYPTI*

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Recombination on the sex-chromosome of *Aedes aegypti* has been studied in male genotypes incorporating the sex-linked translocation T_1 and the meiotic drive gene D from

three different strains (TRINIDAD, BOZO and CARACAS). The introduction of the *D* gene was associated with an increase in recombination between the pseudolinked markers *w* and *blt* in TRINIDAD $DT_1/+$ males, compared with $T_1/+$ males. However, this increase was not seen in BOZO or CARACAS $DT_1/+$ males. Four DT_1/T_1 homozygous lines were obtained after outbreeding and reconstituting the TRINIDAD DT_1/T_1 strain. Heterozygotes ($DT_1/+$) from two out of these four lines did not show the increased $w - blt$ recombination observed in the original TRINIDAD $DT_1/+$ males.

These two heterozygotes, as well as the BOZO and CARACAS $DT_1/+$ males, also failed to show two other properties associated with the basic $DT_1/+$ male: (1) enhanced sex-ratio distortion, (2) high variance in fertility. All these properties probably resulted from a common factor inherited from the JY stock, at a position near *w*, when T_1 and the TRINIDAD *D* were originally combined.

THE EFFECT OF *MONOPLANE* ON EYE DEVELOPMENT IN *DROSOPHILA MELANOGASTER*

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Monoplane (*Mpe*) is a newly discovered, homozygous lethal, dominant wing mutation, resembling *heldout* and *taxi* in that its wings are held horizontally and at 90° to the abdomen. *Mpe* is an X-ray induced translocation between chromosome arms 2L and 3R.

Mpe affects the expression of certain *eyeless* (*ey*) alleles. *Mpe*/+, *ey*/*ey* flies have eye sizes intermediate between that of *Mpe*/+, +/+ and +/+, *ey*/*ey* flies; further, there appears to be none of the variability in size between the two eyes of each fly usually associated with *eyeless*. The mean eye size of *Mpe* flies is not significantly different from that of wild type; however, *Mpe* flies have a greater variance of eye size when compared with wild type flies. *Mpe* also affects the expression of eye size in the other small eye mutant investigated. *Mpe* causes an increase in the eye size of *Glued*, *Bar* and *lozenge* but causes a decrease in the eye size of *Lobe* flies. The smooth shiny appearance of *Glued* and *Lozenge* eyes, due to fused and irregularly shaped facets, is not affected by *Mpe*. The effect of *Mpe* on cell degeneration in the imaginal eye discs of these small eye mutants has been investigated.

A GENE AFFECTING PROTEIN COMPOSITION OF PEA SEED

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The effect of the r_a locus on the proportions of the main groups of storage proteins accumulated in the pea seed has been investigated. A large range of genotypes, seven near-isogenic lines and segregating populations were analysed for the proportion of legumin present in mature seed. In addition to its effect on starch quantity and quality and on the sugar content of the seed, the r_a locus was found to affect the amount of legumin accumulated. The effects of mutation at this locus are compared with those of mutants affecting protein composition in cereals. The location of the structural gene for the 40Kdalton sub-unit polypeptides of legumin has also been identified.

THE EFFECT OF HOMOEOLOGOUS GROUP FIVE CHROMOSOMES OF WHEAT ON RESISTANCE TO TWO COMMON FUNGAL PATHOGENS

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Aneuploid lines in the wheat variety Chinese Spring reveal a negative correlation between dosage of the homoeologous chromosomes 5A, 5B and 5D and the level of adult-plant resistance to yellow rust (*Puccinia striiformis*) and powdery mildew (*Erysiphe graminis tritícii*).

Similar correlations were found for monosomics of these chromosomes in the varieties Poros and Cappelle-Desprez and yellow rust resistance. The magnitude of the chromosome dosage effect differed consistently between homoeologues, due presumably to homoeo-allelic variation. Also, inter-varietal chromosome substitution lines in Chinese Spring showed variable resistance to both diseases for each of the group 5 chromosomes. The negative correlation observed for whole chromosomes of group 5 was not found when lines deficient for the $5B^s$ arm were studied in the varieties Cappelle-Desprez and Hybride de Bersée (Gaines, R., Ph.D. thesis, Cambridge, 1976). This can be explained if increased dosage of the two arms of these chromosomes have opposite effects, the long arms decreasing and the short arms increasing resistance.

IN SITU HYBRIDISATION OF DNA SEQUENCES TO MEIOTIC CHROMOSOMES IN HYBRIDS BETWEEN *AEGILOPS* AND *SECALE*

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The development of the technique of *in situ* hybridisation of ^3H labelled cRNA copies of DNA sequences cloned in bacterial plasmids has provided a valuable method for the identification of chromosomes. Using DNA sequences cloned from the rye genome it has been possible to analyse the meiotic pairing behaviour in *Aegilops* × Rye hybrids. In the F1 hybrids between two diploid species, *Ae. comosa* or *Ae. caudata*, and *S. cereale* cv "Petkus Spring", bivalent formation was mostly of the *Aegilops*/Rye type, although a low frequency of Rye/Rye and *Aegilops*/*Aegilops* bivalents was also found. On the other hand, in the F1 hybrids between the tetraploid *Ae. columnaris* and Rye, bivalents and occasional trivalents were predominantly limited to the *Aegilops* chromosomes.

ALTERNATIVE STATES OF SEQUENCE ORGANISATION ASSOCIATED WITH S-TYPE CYTOPLASMIC MALE STERILITY IN MAIZE

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Mitochondria from maize lines which are male sterile because they contain type S cytoplasm have two small additional DNAs containing approximately 6600 and 5500 base pairs respectively (Pring *et al.*, *Proc. Nat. Acad. Sci., U.S.A.*, *74*, 2904-2908). These DNAs are absent from normal male fertile lines. Restriction fragments of these two DNAs have been cloned in bacterial plasmids and used to show that homologous sequences in the two DNAs are found integrated in main band mitochondrial DNA from male-fertile cytoplasms. The results show that these sequences occur in integrated and non-integrated forms and that male sterility is associated with the occurrence of the non-integrated form.

NUCLEOTIDE SEQUENCE AMPLIFICATION AND TRANSLOCATION IN HIGHER PLANT CHROMOSOMES

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Members of several different families of repeated sequences in the wheat genome have been cloned in bacterial plasmids. These clones have been used to study the organisation of these families in the wheat genome and in the genomes of closely related species. The results provide further evidence for the frequent amplification and translocation of short segments of DNA during chromosome evolution.

MUTATION IN DYING POPULATIONS OF *ESCHERICHIA COLI*

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Substrate-accelerated death was studied in lactose-limited cultures of strains *Escherichia coli* WP2 *trp*$^-$ and *E. coli* WP2 *trp*$^+$. During starvation of *E. coli* WP2 *trp*$^-$ in saline-phosphate buffer pH 7·4 containing lactose the total viable count decreased linearly while the number of *trp*$^+$ revertants increased; a similar phenomenon was observed in cultures of histidineless *E. coli* (Ryan, *J. Gen. Microbiol.*, *21*, 530, 1959). Addition of 7·5 mM-cyclic AMP to the starvation medium alleviated the death of the *trp*$^-$ cells but did not prevent the increase in the number of *trp*$^+$ revertants. Starvation of *E. coli* WP2 *trp*$^+$ did not result in death, suggesting that the level of cyclic AMP in the *trp*$^+$ revertants is higher than in the *trp*$^-$ cells. As addition of inhibitory concentrations of benzyl penicillin or nalidixic acid did not affect the viable counts it indicates that neither cryptic growth nor DNA replication took place. Determinations of β-galactosidase activity provided further evidence that cryptic growth did not occur. Reasons for this increase in the number of *trp*$^+$ revertants during the lactose-accelerated death of *trp*$^-$ cells will be considered.

8-AZAGUANINE RESISTANCE IN *SCHIZOSACCHAROMYCES POMBE*

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8-azaguanine resistant mutants have been reported in a number of organisms and, in most cases, resistance has been associated with the loss, or reduced activity, of the salvage pathway enzyme, HGPRT. In *Sch. pombe*, however, *aza 1* mutants have altered activity of the first enzyme of *de novo* purine biosynthesis, resulting in a lowering of its feedback control by the end products of the pathway (Heslot *et al.*, *C.R. Acad. Sci.*, *263*, 57, 1966; Nagy, *Biochim. biophys. Acta*, *198*, 471, 1970). Genetical analysis indicates that at least two classes of spontaneous and UV-induced *aza* mutants occur in prototrophic strains of *Sch. pombe*. Although most *aza* mutants produce white colonies on yeast extract medium, a small minority form red pigmented ones. The former class are mitotically stable whilst the latter give rise to occasional white colonies. Ascus dissection from appropriate crosses suggests that colony pigmentation is not simply a pleiotropic effect of mutation to azaguanine resistance but rather that antibiotic resistance and colony pigmentation are coded for by two independent and unlinked genes (McAthey, *Molec. gen. Genet.*, *149*, 239, 1976). Red pigment production, which can be alleviated by increasing the concentration of biotin in the medium, is thought to result from a partial blockage at the *ade 7*—dependent step of the *de novo* pathway. Pigmentation analysis of *aza* mutants isolated in auxotropic strains blocked at various steps in this pathway will be discussed.

CHLORAMPHENICOL RESISTANCE IN *SCHIZOSACCHAROMYCES POMBE*

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Seven previously isolated strains of *S. pombe*, *cyh2*, *cyh3*, *cyh4*, *tri3*, *tri4*, *ani1*, and *ani2* were shown to be cross resistant to the three ribosomal inhibitors cycloheximide, trichodermin and anisomycin (Ibrahim and Coddington, *Heredity*, *37*, 179, 1976; Berry *et al.*, *Molec. gen. Genet.*, *167*, 217, 1978). The simplest explanation for the acquisition of such resistance to chemically unrelated drugs is that the mutation leads to a general alteration in membrane permeability, resulting in decreased drug uptake. If this were true, then one might also expect these strains to show cross resistance to drugs acting at other sites in the cell, *e.g.*, the mitochondrion. All the above strains were tested for growth on solid media containing 160 mM glycerol as carbon source, plus either antimycin, tetracycline or chloramphenicol at levels which

completely prevented wild-type growth. Two strains, *cyh3* and *cyh4* were resistant to all three drugs. Uptake of [^{14}C]-chloramphenicol by wild type, *cyh3*, and *cyh4* was measured at 5 mM and 10 mM external concentration. Both the *cyh3* and *cyh4* strains showed a decreased uptake when compared to the wild type, the effect being most marked at the higher concentration. This suggests that for chloramphenicol, at least, reduced membrane permeability could be the reason for the resistance shown by these two strains.

INHERITANCE AND STABILITY OF HYBRID YEAST-*ESCHERICHIA COLI* PLASMIDS IN *SACCHAROMYCES CEREVISIAE*

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Saccharomyces cerevisiae can now be transformed using various yeast-bacterial hybrid plasmids (Hinnen *et al., Proc. Natl. Acad. Sci., U.S.A., 75*, 1929-1933, 1978). Previously it had been reported that if such vectors contained endogenous yeast plasmid DNA sequences, they gave highly unstable yeast transformants (Struhl *et al., Proc. Natl. Acad. Sci., U.S.A., 76*, 1035-1039, 1979). We would like to report on highly stable transformants produced by using the chimaeric plasmid pJDB219 (Beggs, *Nature, 275*, 104-109, 1978). pJDB219 was constructed by J. D. Beggs and consists of the yeast plasmid (Scp1-L form), a 1·2 kb yeast nuclear DNA fragment carrying the LEU2$^+$ gene and PMB9, a derivative of the *E. coli* Co1E1 plasmid. MC16 (α, ade 2·1, leu 2·3, his 4·712, SUF2, 2 μm DNA$^+$) was used as the recipient strain and various LEU$^+$ transformed clones of MC16 were analysed for inheritance of leucine prototrophy and for molecular changes in the pJDB219 after long-term growth on selective medium. In most transformed clones the pJDB219 remained unaltered. A new LEU$^+$ plasmid, smaller than pJDB219 was generated in two clones and in all the clones except one, the endogenous 2 μm plasmid had been lost in favour of the hybrid pJDB219.

REVERSION OF INDUCED MUTATIONS OF THE CYTOPLASMICALLY-INHERITED [psi$^+$] DETERMINANT

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An extrachromosomal determinant, [psi], enhances the efficiency of suppression by ochre suppressors such as SUQ5 (Cox, *Heredity, 20*, 505-521, 1965). Various agents induce mutation from [psi$^+$] to [psi$^-$]; these include nuclear mutagens (EMS, NTG and UV) and a series of agents which have no effect on nuclear genes or on *petite* mutagenesis (guanidine hydrochloride, dimethyl-sulphoxide, methanol and KCl) but cause 100 per cent loss of the [psi$^+$] phenotype. Reversion studies on [psi$^-$] mutants induced with these agents reveal both revertible and seemingly non-revertible [psi$^-$] mutants induced by GuHCl, DMSO or NTG are of the former type; those induced with 2 M KCl or 12·5 per cent methanol of the latter type. [psi] is of particular interest as the phenotype is "cured" by agents not usually found to be mutagens (*e.g.*, methanol) but not by agents which cure bacterial plasmids, *e.g.*, ethidium bromide and acridine dyes.

EFFECTS OF SELECTION ON GENETIC VARIABILITY

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Selection affects genetic variability in two ways, by inducing linkage disequilibrium which causes correlations between loci and by causing changes in gene frequencies. The theory of these two processes is briefly reviewed, with particular regard to the factors which may be responsible for maintaining genetic variability in natural populations.

EFFECTS OF DISRUPTIVE SELECTION ON GENETIC VARIABILITY IN *DROSOPHILA*

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Bulmer (*Amer. Nat.*, *105*, 201, 1971) has shown that if a trait is determined by an infinite number of loci, selection changes the genotypic variance by generating linkage disequilibrium. With a finite number of loci the genotypic variance is further affected by gene frequency changes but with disruptive selection these can be ignored relative to the effect of positive linkage disequilibrium. The following experiment with *Drosophila melanogaster* was designed as a test of Bulmer's theory.

The highest (H) 10 per cent and lowest (L) 10 per cent of males and females on abdominal bristles were selected and mated in the following way: H × H, H × L, L × H, L × L, with 8 pairs in each, for 3 generations and heritability (h^2) was estimated each generation. This was followed by a period of 4 and 6 generations of relaxation and h^2 was reestimated. The experiment was replicated twice and the pooled estimates of h^2 from the regressions of offspring on mid-parent together with results predicted from theory were as follows:

Generation	0	1	2	7	9
Observed	0·37 ± 0·04	0·63 ± 0·04	0·69 ± 0·05	0·44 ± 0·07	0·47 ± 0·06
Predicted	(0·37)	0·56	0·65	0·47	0·42

Results are in good agreement with theory, the pattern of change being consistent with the expectation that changes in the genotypic variance are mainly due to linkage disequilibrium.

THE MAINTENANCE OF AN INVERSION POLYMORPHISM IN SEAWEED FLIES

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All populations of the seaweed fly, *Coelopa frigida*, are polymorphic for the $\alpha\beta$ inversion on chromosome I. In spite of this fly being exposed to considerable environmental variation (availability and composition of food, climate, etc.) the inversion polymorphism is held tightly at an equilibrium value. One of the selective forces acting is heterokaryotypic advantage in the viability of larvae and pupae. In high density laboratory populations the viability of each homokaryotype (relative to the heterokaryotype) can be lower than 30 per cent. The availability of food to natural populations of *Coelopa* is, to a large extent, coordinated with the lunar cycle. To have a generation time that is too fast is just as much a disadvantage as to have one that is too slow. The fact that heterokaryotypes have an intermediate generation time, constitutes a second selective force stabilising the polymorphism. There is evidence that flies with the lowest larval viability have an advantage in mating success, at least in laboratory cultures. This may also play a role in what appears to be a complex system of balancing selective forces.

SELECTION ON RECOMBINATION RATES IN HIGHER ORGANISMS

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The results of some artificial selection experiments on recombination rates in higher organisms are reviewed. The light shed by these experiments on the properties of genetic modifiers of recombination rates is discussed. The implications for understanding the response to selection on recombination rates in natural populations are examined.

MATERNAL PHENOTYPE OVERSPILL: AN ANALYSIS OF VARIABLE PENETRANCE AND EXPRESSIVITY IN THE *SMALL EYES* MOUSE MUTANT

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The *Small eyes* (*Sey*) mutation in the mouse affects the glycosylation of collagen, producing a range of gross ocular abnormalities, including small eyeballs, tissue degeneration and lens cataract (Clayton and Campbell, 1969, *J. Physiol., Lond.*, *198*, 74; Pritchard and Clayton, 1974, *Exp. Eye Res.*, *19*, 335). It is a homozygous-lethal with variable dominance status and expressivity (Roberts, 1967. *Genet. Res. Camb.*, *9*, 121), depending partly on genetic background. Matings between *Sey* and normal mice produce non-Mendelian ratios of phenotypes, related to maternal genotype and age. Young, normal (+/+) C57B1/Fa females mated to heterozygous (*Sey*/+) males with the C57B1/Fa genetic background, produce a significant excess of offspring of normal phenotype, whereas young, female *Ju*/Fa *Sey*/+ heterozygotes and normal *Ju*/Fa males produce a significant excess of *Sey* phenotype young. There was no evidence of selective mortality. The reciprocal matings give the expected (1 : 1) ratio of phenotypes, as do all matings with mothers older than thirty-seven weeks (Pritchard, 1974, *Heredity*, *33*, 143). Breeding tests reveal an inconsistency between phenotype and genotype, indicating an "overspill" of maternal phenotype into the progeny, so that some mutant mice appear normal, while genotypically normal mice can resemble *Sey*. Physiological "overspill" of maternal phenotype is also indicated by analysis of expressivity, the genotypically mutant progeny of mutant mothers being more severely affected than those of normal mothers. However, the results of embryo transplantation experiments did not support the concept of physiological "overspill".

CYTOGENETIC STUDIES IN A TRISOMY 8 MOSAIC HUMAN MALE ASCERTAINED THROUGH INFERTILITY

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Trisomy 8, in mosaic or non-mosaic form, is an extremely rare chromosomal condition in man. Liveborn subjects usually present with mental retardation, bone and joint anomalies and a variety of other physical anomalies (Riccardi, *Birth Defects: Orig. Article Ser.*, *XIII*, 171, 1977). The mental retardation associated with the condition is, however, usually moderate compared to that found in other human autosomal trisomic conditions. The present report describes a trisomy 8 mosaic male subject with normal IQ and near-normal phenotype, ascertained through infertility. Chromosome studies made on peripheral blood lymphocytes showed a pure trisomy 8 constitution while cultured skin fibroblasts revealed 46, XY/47, XY+8 mosaicism. Meiotic studies showed that the extra No. 8 chromosome, found in somatic tissues, was missing from the germ line. The testicular histology indicated a germ cell maturation arrest in 80 per cent of seminiferous tubules and the patient was severely oligospermic. Biochemical studies to assay levels of glutathione reductase, a red cell enzyme, the gene for which resides in chromosome 8, showed increased levels in the trisomy 8 patient compared with controls.

ROLE OF TRANSPOSABLE ELEMENTS AND SITE-SPECIFIC RECOMBINATION IN THE EVOLUTION OF MICROBIAL GENOMES

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Faithful replication of a non-redundant genome and homologous recombination between resulting daughter genomes cannot generate chromosome rearrangements such as duplication, deletions, inversions and translocations. In contrast, transposable elements can effect rearrangements in a number of ways. Firstly, by their ability to insert at a number of loci within a

genome, and to be present in more than one copy per cell, they provide regions of "portable DNA homology" upon which homologous recombination systems can act to produce genome rearrangements. Moreover transposable elements can directly indicate *illegitimate recombination* events that generate rearrangements, whose end points are usually close to the insertion point of a given element. In *Escherichia coli*, transposable elements are not responsible for all illegitimate recombination events. For example, site-specific *recA*-independent recombination systems that are specified by some extrachromosomal elements, may have contributed to the evolution of these extrachromosomal genomes.

PLASMIDS, TRANSPOSONS AND BACTERIOPHAGE—DO THEY MAKE A NONSENSE OF BACTERIAL TAXONOMY?

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It is now generally accepted that plasmids and some phages play an important role in the survival of many bacterial populations. Transposons probably also play a part, but less is known about the effects of these genetic elements. The presence of all these genetic entities in bacterial populations, or in some members of populations, is often manifested by changes in the observable characters of the bacteria. These observable characters are frequently those used by the bacterial taxonomist in the pursuit of his science. Therefore, because it is known that plasmids, transposons and phages are found and can be transferred between a wide range of bacterial taxa (often designated as separate species, genera and even families by bacteriologists), it has become fashionable amongst molecular biologists to imply or state that "these recent genetic observations have made a nonsense of bacterial taxonomy". It is not clear from statements of this kind whether the "taxonomy" being referred to is bacterial classification or identification. The implications of the effects of plasmids, transposons and phages for the bacterial taxonomist will be discussed in this context. Claims are also made that the study of the effects of transposable elements throws light on bacterial evolution, but rarely is any distinction made between long term evolution (phylogeny), and short term changes—adaptations to specific environments.

ON THE EVOLUTION OF TRANSPOSABLE ELEMENTS

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The transposon Tn501 codes for resistance to mercuric ions, and Tn1721 codes for amplifiable resistance to tetracycline. The ends of Tn501 are an inverted repeat of 38pb which contains an *Eco*R1 site; the element is flanked by a 5bp direct repeat of host DNA. Tn1721 contains repeated sequences that contain an *Eco*R1 site: the parts of this sequence, and the sequences immediately adjacent to it that have been determined are identical to the corresponding sequences in Tn501. Furthermore, hybridisation by Southern blotting shows that there are other homologous regions within the transposons. The inverted repeat of Tn501 is at least 50 per cent homologous with the reported sequences of the inverted repeats of Tn3, $\gamma\delta$, and the insertion sequence in pSC101. All of these elements are flanked by a direct repeat of 5bp of host DNA. These similarities in phenotypically diverse transposable elements suggest that all complex transposable elements may have descended from the same ancestor.

THE SITE-SPECIFIC RECOMBINATION SYSTEM SPECIFIED BY THE Ap^r TRANSPOSONS Tn*1/3*

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The closely related ampicillin-resistant (Ap^r) transposons Tn*1/3* specify a *rec*A-independent site-specific recombination system. The determinants include a gene specifying a *trans*-acting 19,000 molecular weight protein and a site at which this protein is thought to act. This

recombination system is used to resolve cointegrate intermediates in transposition. In the absence of transposition it can also act to recombine Tn*1/3*—containing sequences. This recombination system acts much more efficiently than *rec*A-dependent homologous recombination on the same sequences. The nature of this recombination process and the implications of cointegrate formation and *rec*A-independent site-specific recombination in genetic rearrangement will be discussed.

THE INSERTION SEQUENCE IS2—A MOBILE PROMOTER IN *ESCHERICHIA COLI* K-12

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Plasmids have been constructed that carry the galactose operon region of the *E. coli* chromosome (Ghosal A. and Saedler H. (1977) *Molec. gen. Genet., 158,* 123). Integration of the insertion sequence IS1 in both orientations into the *galOP* region and the subsequent IS1-driven deletion of the galactose operon promoter, yields plasmids in which IS1 is still present and the galactose operon is uncoupled from a promoter. Subsequent selection of Gal^+ revertants yielded several independent isolates that carried 1400 base pair insertions into IS1. Southern transfer experiments proved these insertions to be IS2 in orientation II, which is known to carry a promoter (Saedler, H., Reif, J., Hu, S. and Davidson, N. (1974). *Molec. gen. Genet., 132,* 265). There seemed to be a hotspot for IS2 integration near one end of IS1.

SEQUENCE REARRANGEMENTS AT A DELETION ENDPOINT WITHIN IS2; A WAY FOR THE CREATION OF COMPLEX GENETIC SIGNALS IN *E. COLI*

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The analysis of unstable revertants of an IS2 mutant of the galactose operon of *E. coli* led to the discovery of a new pathway which, by DNA synthesis and DNA sequence rearrangements, eventually leads to the creation of complex genetic signals, for example, promoters. The DNA sequences of the Gal^+ revertants and their Gal^- segregants will be presented. A model that explains the creation of new DNA sequences by the action of DNA polymerase will be discussed. The observed sequence rearrangements may be of general importance and may have played a role in the evolution of the bacterial chromosome in the past and may do so even today.

THE TRANSPOSITION OF Mu FROM A PLASMID SUBSTRATE

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The derivative of bacteriophage Mu, MupAp1, which confers resistance to the antibiotic ampicillin has been inserted into the plasmid Col E1 derivative pML2. This hybrid plasmid, pSU1, has then been used to study the transposition of Mu from a plasmid substrate.

(1) When transposition of MupAp1 is selected from pSU1 to the sex factor R388, cointegration of the two plasmids frequently occurs. The structure of these Mu induced cointegrates was examined by restriction analysis and by observation of their behaviour when introduced to Rec^+ cells with the conclusion that they contain the two plasmids separated by two copies of MupAp1 oriented in direct repeat with respect to each other.

(2) When the plasmid DNA content of cells harbouring pSU1 is examined on agarose gels at different times after thermal induction of the prophage it can be seen that the pSU1 bands gradually disappear as induction proceeds. This loss of free plasmid DNA during induction may represent cointegration of pSU1 with the bacterial chromosome.

These two results suggest that the first event that occurs on induction of a Mu containing plasmid is the cointegration of this DNA with either the chromosome or another plasmid in the cell.

STUDIES ON BACTERIOPHAGE Mu INDUCTION AND TRANSPOSITION

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The temperate bacteriophage Mu transposes its DNA during the lytic cycle into new sites on the host genome. Some models have been proposed to explain the transposition process and they take into account a lot of the experimental evidence. Nevertheless we still need to develop systems in which several of the predictions of these models can be tested. We have previously constructed a plasmid, pSU1, containing the whole DNA of the inducible phage MupAp1, a derivative of Mucts which includes a region coding for ampicillin resistance. To study the process of transposition of Mu from this plasmid in more detail we set out to prepare derivatives of pSU1 with mutations in regions probably involved in transposition. The early region of Mu and, in particular, the A and B genes, have been assigned roles in this mechanism. Thus, we have obtained plasmids containing amber mutations in either A or B. An X derivative, containing a Tn*9* insertion into the early region was also prepared. We are examining the properties of the plasmids carrying these A, B or X mutations both by agarose gel electrophoresis throughout the heat induction process and by genetic transposition experiments, the results being compared to those obtained with pSU1.

EVOLUTIONARY ASPECTS OF NITROGEN FIXATION IN KLEBSIELLA

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The ability of Klebsiella to fix atmospheric nitrogen depends on at least 14 genes, located close to the *his* operon (Dixon *et al.*, *Molec. Gen. Genet.*, *157*, 189, 1977; MacNeil *et al.*, *J. Bacteriol.*, *136*, 253, 1978). These genes have been identified by intensive work on a particular Klebsiella strain M5a1, and little attention has been given to other strains. However, surveys of Klebsiellas from diverse sources, by ourselves and others (refs. in Chambers and Silver, *J. clin. Microbiol.*, *6*, 456, 1977) gave the surprising result that about half the many wild strains tested are unable to fix nitrogen. This fact raises interesting evolutionary questions, and several hypotheses to explain it could be put forward. (1) Nif^+ and Nif^- bacteria form two distinct groups, which separated early in Klebsiella history. This is unlikely, since no other biochemical or habitat differences appear to correlate with ability to fix N_2. (2) Erosion of the *nif* region by mutational decay is balanced by natural selection favouring Nif^+ bacteria. If so, the selection advantage must be very weak or only occasionally expressed. (3) The *nif* genes were spread by a plasmid derived from another source, and could be integrated near *his*. (4) The *nif* DNA region can be excised, and possibly transposed between strains. As a first step in testing these hypotheses, we have transferred a plasmid (pRD1) carrying the complete *nif* region, and derived plasmids with one of the seven genes *nif B*, *A*, *F*, *E*, *K*, *D* and *H* inactivated (Dixon *et al.*, *op. cit.*) into eleven wild non-fixing strains. pRD1 made all these strains Nif^+, but the mutant plasmids left them all Nif^-. This suggests that probably the whole *nif* DNA region was inactive or missing in these strains, and makes hypothesis (2) unlikely.

BIOLOGICAL NITROGEN FIXATION: DIVERSE ORGANISMS WITH COMMON BIOCHEMISTRY AND GENETICS

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The ability to reduce or "fix" atmospheric nitrogen to ammonia is an exclusively prokaryotic property found amongst cyanobacteria, Gram positive and Gram negative bacteria. Yet within any particular genus some species can fix nitrogen but others cannot. Why is the possession of this important attribute at the same time so sporadic but taxonomically so

wide-ranging? One attractive hypothesis states that the nitrogen-fixation (*nif*) genes are, or have been, part of a transposon and that the *nif* genes have spread from some unknown source *via* transposition to a wide range of bacteria. Evidence in favour of such a model will be discussed. One of the best-known nitrogen fixing genera is *Rhizobium*, the bacterium responsible for fixation in legume root nodules. The rhizobia can be divided loosely into two groups. One of these comprises slow-growing strains that tend to nodulate tropical legumes; the other grows more rapidly and nodulates temperate legumes. Over and above this division, rhizobia are further allocated to species according to their host range. For example, *R. leguminosarum* is defined by the ability of members of this species to nodulate *Pisum*, *Lens*, *Lathyrus* and *Vicia* but not (for example) *Trifolium* or *Lupinus*. In some strains of *Rhizobium* the genes for host range are plasmid-linked and the nodulation specificity of one *Rhizobium* species can be altered by the introduction of a plasmid from a different species. We will discuss the consequences of this transfer of host range and the interaction between plasmids that carry genes that determine the ability to nodulate different legume species.

AN OVERLAP BETWEEN GLUTAMINE AND METHIONINE TRANSPORT SYSTEMS IN *SALMONELLA TYPHIMURIUM*

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Glutamine is transported into *Salmonella typhimurium* by two systems, one with a high affinity and one with a low affinity for glutamine (Ayling and Betteridge, *Heredity*, *35*, 436-437, 1975). Two classes of mutants lacking the high-affinity system for glutamine have been previously described, *glnP* (Betteridge and Ayling, *Molec. gen. Genet.*, *138*, 41-52, 1975) and *glnH* (Kustu *et al.*, *J. Bact.*, *138*, 218-234, 1979).

Three lines of evidence indicated that the low-affinity glutamine transport system shares some of the components of a previously described high-affinity transport system for methionine (Ayling and Bridgeland, *J. gen. Microbiol.*, *73*, 127-141, 1972). First, *metP* mutants, which lack the high-affinity methionine system, show reduced activity of the low-affinity glutamine system. Second, uptake of glutamine by the low-affinity glutamine system was inhibited by L or D methionine. Third, *metP glnP* strains grew more poorly on glutamine as the sole nitrogen source than *glnP* strains. However, glutamine significantly inhibited the transport of only D, but not L methionine. The high-affinity methionine system may have at least two components, with different specificities for L and D-methionine and glutamine. *metP* mutations prevent growth of methionine auxotrophs such as *metB* on D-methionine. Two revertants which were isolated from *metB23 metP760* on D-methionine had regained partial activity of the *metP* system. Growth of these strains on D-methionine was inhibited by glutamine (unlike the *metB* strain); this may be explained by the fact that glutamine inhibited the transport of D-methionine. Studies on periplasmic proteins from these mutants are in progress.

THE RESPONSE OF THE CYANOBACTERIUM *GLOEOCAPSA ALPICOLA* TO DNA DAMAGE BY FAR ULTRAVIOLET IRRADIATION

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Preliminary evidence for the presence of an excision system for DNA repair in the unicellular cyanobacterium *Gloeocapsa alpicola* has recently been reported by Williams, Lambert, O'Brien and Houghton (*Photochemistry and Photobiology*, *29*, 543, 1979). This evidence was based on an observed decrease in the photoreversible sector during dark liquid holding of a far ultraviolet light irradiated culture. Split UV dose experiments indicated that light dependent repair remained operational during dark liquid holding. Studies with chloramphenicol and rifampicin indicated that inducible functions played only a minor role. The present communication extends these observations with a report of investigations into the biochemical mechanisms responsible for the loss of photoreversibility.

THE CONSTRUCTION AND PROPERTIES OF A SMALL RECOMBINANT PLASMID CONTAINING THE ORIGIN OF TRANSFER OF THE SEX-FACTOR F

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Transfer of the F-plasmid between conjugating *E. coli* cells has been hypothesised to require endonucleolytic cleavage of a specific strand of DNA at a specific site. This site, the origin of transfer (*oriT*) has been mapped between cleavage sites of restriction endonucleases *Sal*I and *Bgl*II to the left of the transfer operon. This *Sal*I-*Bgl*II fragment, of 590 base pairs, was cloned into pBR322 and the properties of the recombinant plasmid (pED806) studied. The endonuclease restriction map of pED806 was as predicted, and the plasmid was transferred at high frequency from cells which also contained Flac. Although pED806 showed stable inheritance in the absence of Flac, the presence of the sex factor resulted in rapid segregation of the recombinant. The instability of pED806 could be correlated with the presence or absence of certain Flac *tra* cistrons, and was considerably more marked in a *recA* background. The F DNA carried by pED806 does not, as far as is known, code for any transfer proteins. However, about 10 per cent of the transconjugants from a cross between a *recA* Flac pED806 donor and a *recA* recipient had inherited only the recombinant plasmid. Therefore this result supports the hypothesis that recircularisation of transferred DNA occurs at *oriT* in a *recA* independent process, and in the absence of transcription of known F *tra* cistrons. The implication is that if any F *tra* proteins are required for recircularisation, they are transferred from the donor with the DNA.

A REGION ON RP4 AFFECTING ITS STABLE INHERITANCE

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It has proved possible to construct derivatives of the wide host-range plasmid RP4 consisting of a contiguous region less than half the original molecule. Within this region three fragments have been shown, in the related or identical plasmid RK2, to be necessary for stable plasmid replication and maintenance in *Escherichia coil*. However, we have isolated deletions in the non-essential region of RP4 (close to the Km^R determinant) that affect the stable inheritance of these derivatives in *Pseudomonas aeruginosa* or *E. coli*. Some of the deletions produce dramatic instability but the effect is reversible by deletion of an adjacent sequence. We have cloned the regions involved onto a plasmid vector to study their effect *in trans* and determine the functions involved.

INVESTIGATION OF THE RELATIONSHIP BETWEEN PLASMID MEDIATED IRON UPTAKE AND COLICIN V

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Many strains of *Escherichia coli* isolated from cases of bacteraemia of humans and domestic animals carry plasmids specifying synthesis of colicin V (Smith, H. W., 1974, *J. Gen. Microbiol.*, *83*, 95-111). Recently it has been shown that these ColV plasmids encode a novel iron-uptake system which is an important component of the virulence of invasive strains of *E. coli* (Williams, P. H. and George, H. K. 1979, *Plasmids of Medical, Environmental and Commercial Importance*, Timmis, K. N. and Puhler, A., editors, 161-172). In conditions of iron stress, a number of proteins of the outer membrane of *E. coli* are induced, some of which are known to be involved in the uptake of iron by various routes. However, one whose role in iron

uptake has not been established is the receptor protein for colicin V. Therefore, the possibility that there exists a mechanistic relationship between colicin V and the ColV plasmid mediated iron-uptake system has been investigated. Mutants of *E. coli* K-12 that are defective in the synthesis of the iron chelating molecule enterochelin cannot grow in medium containing a low level of iron (2 μM) unless sodium citrate is added to complex available iron for active uptake. The presence of ColV plasmids in an enterochelin deficient mutant obviates the requirement for added citrate. To determine the relationship between synthesis of colicin V and the production of the plasmid coded iron chelator, we have analysed clones of mutagenised ColV plasmid-carrying bacteria for simultaneous loss of citrate independence and colicinogenicity. To investigate the correlation of colicin killing activity with plasmid determined iron uptake, we have tested the capability of ColV plasmids to reverse the citrate dependence of colicin resistant and colicin tolerant derivatives of enterochelin deficient mutants. Our results will be discussed in relation to the involvement of colicin V in the virulence of ColV plasmid-carrying invasive strains of *E. coli*.

THE GENOME STRUCTURE OF T1 AND RELATED PHAGES

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The genome of phage T1 has a molecular weight of 31×10^6, a terminal redundancy of 6·5 per cent and is present in phage particles as three cyclic permutations (Gill and MacHattie, *J. Molec. Biol., 104*, 505, 1976). These derive from the headful packaging of 1·065 genome equivalent lengths from a concatemeric DNA substrate. A physical map of the T1 genome has been constructed from restriction enzyme digests, principally with Bgl.I and Bgl.II. This map incorporates the features described above and identifies the site at which headful packaging is initiated on the DNA concatemer. The restriction patterns of various T1 stocks, the related phage D20 and hybrid phages have been compared. From these results the inter-relationships among these phages have been analysed.

EVOLUTION OF THE T7-LIKE PHAGE INVOLVED RECOMBINATION AT SITES BETWEEN GENETIC MODULES IN T7-LIKE AND T3-LIKE ANCESTORS

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It has long been realised that recombination plays an important part in evolution because it increases genetic variation. We shall present evidence from restriction enzyme analysis that the T7-like phage (represented by T7, T3, ØI, ØII, H, W31 and Cro) which are closely related and can undergo intertypic recombination have evolved by a process which included recombination between T7-like and T3-like ancestors. The variability generated by recombination has apparently been significant in the evolution of this phage group. The recombination events in question have a non-random distribution, and this is also found for the unselected recombination events which occurred during the formation of a group of T7/T3 hybrids made by Hausmann which we have also analysed. In the cases of both natural and artificial phage the pertinent recombination events occurred more frequently than expected by chance in regions which separate *modules* of genes, genes within a module having related functions. These observations provide evidence for the idea proposed by Herskowitz and Botstein (*Nature, 251*, 584, 1974) that recombination between modules is advantageous. Such events increase genetic variation without breaking combinations of genes whose products must interact in a highly specific way. As pointed out by Fisher (*The Genetical Theory of Natural Selection*, Clarendon Press, Oxford, 1930) there will be selective pressure to increase and maintain linkage between genes in such modules. It now appears that in some systems there may also be pressure to decrease linkage between modules (*Bacteriol. Rev., 40*, 552, 1976).

THE EVOLUTION OF THE TRANSCRIPTIONAL SYSTEMS OF THE T7-LIKE BACTERIOPHAGES

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The evolution of transcriptional systems composed of several elements including RNA polymerases, promoters, terminators and accessory control factors is poorly understood. Only recently has sufficient structural and functional information become available to permit useful comparisons between different systems. The RNA polymerases and promoters of the phages T7 and T3 and of their host *E. coli* have been studied in such detail and there are now indications as to how they might have evolved. We shall review the evidence including some new deductions from DNA sequences, promoter cloning and T7/T3 hybrids showing the relationships between the three systems. It has been known for some time that the two phage systems are similar, but there is new evidence that the bacterial and the phage systems may be related. The phage systems include an RNA polymerase and several promoters. The T7 promoters (unlike the *E. coli* promoters) are nearly identical to each other indicating tight structural constraints on the RNA polymerase promoter interactions. No T3 promoter has been sequenced but there is evidence that T3 promoters are very similar to each other. If this is true then the T7 and T3 systems have diverged and in a coordinate manner. It is simplest to consider such divergence as having started with mutations in the RNA polymerase gene which affected the interaction between the RNA polymerase and the promoters. This would have enabled a new range of promoter mutants to survive, perhaps contributing to a selection pressure for them. At a later stage recombination between diverged RNA polymerase genes as between those from T7 and T3 may have increased the rate of divergence.

DEFECTIVE LAMBDOID PROPHAGES IN *E. COLI* STRAINS

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Three regions of the *E. coli* K12 (λ^-) chromosome share homology with λ and related phage DNAs. At least two of these (the *rac* and *qsr'* regions) contain blocks of genes that may functionally replace analogous genes carried by λ and are therefore thought to be lambdoid prophages. The genomes of the Rac and *qsr'* prophages are apparently organised in a similar fashion to the λ prophage but, by analogy with λ are probably deleted for structural (head and tail) genes suggesting that both prophages are defective. Suppression of the Rec phenotype of *recBC* strains of *E. coli* K12 may result from constitutive expression of the Rac prophage gene *recE*. Experiments with several other *E. coli* and related enterobacterial DNAs suggest that defective (and probably multiple) lysogeny for lambdoid prophages is a common feature of the *Enterobacteriaceae* phage.

THE EVOLUTION OF LAMBDOID PHAGE GENOMES

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Heteroduplexes formed between the DNA molecules of pairs of lambdoid phages, have in general been found to be composed of distinct homologous and non-homologous regions, when examined by electron microscopy. The lengths of the two strands in the latter regions usually differ. Since the lambdoid classification is based on the possession of a number of common properties, and since viable hybrid molecules can be formed by recombination, it has been proposed that the phages have evolved from a common ancestor. We have asked the following questions. 1. Is each genome divided into discrete segments which are either homologous or non-homologous with the corresponding segments in other phages? 2. How many copies exist for each segment? 3. Could the phages have evolved directly from a common ancestor, or must

there have been exchange of segments between phages? We have sought to answer these questions by forming all the possible heteroduplexes between λ, 434, 82, PA2, 21 and 424. We have reached the following conclusions. 1. The genome is segmented. 2. The number of copies for most segments is likely to be small. 3. Most of the diversity can be explained by a scheme of direct evolution from a common ancestor, but there must have been at least one exchange.

VARIATION OF PLASMIDS WITHIN AN INCOMPATIBILITY GROUP

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The classification of plasmids into incompatibility groups has been useful in studying their epidemiology. Plasmids within an incompatibility group cannot coexist stably within the same cell line and usually show much DNA homology and determine serologically related pili (Datta, 1979). Plasmids belonging to the I and H complexes are exceptional. Group I was so called because it included ColI plasmids. The examples first described were incompatible with one another and produced serologically-related pili whose tips acted as receptors for phage Ifl (Meynell, Meynell and Datta, 1968; Meynell and Lawn, 1969). It was assumed that any plasmid conferring sensitivity to Ifl must have "I" pili, thus all such plasmids were classified into "I" groups, but not all were incompatible with one another (Grindley, Humphreys and Anderson, 1973; Hedges and Datta, 1973). The tips of pili, however, are relatively non-specific phage receptors (Bradley and Rutherford, 1975). The pili of I plasmids fall into two distinct serotypes. The first includes plasmids of incompatibility groups Ia, Iγ, B and K, and the other is represented by I2 and Iδ plasmids (D. Bradley personal communication). IncB plasmids show considerable DNA homology with Iα plasmids (Falkow *et al.*, 1974) and, although their pili are serologically like Iα ones, they confer no Ifl sensitivity. The fact that IncK plasmids determine pili of the Iα serotype is unexpected since IncK was not previously thought to have any connection with the I complex. H1 and H2 plasmids are incompatible yet have little DNA homology (Grindley *et al.*, 1973), and a third IncH subgroup, H3 has been found, incompatible with the others, but sharing little homology with either H1 or H2 (Roussel and Chabbert, 1978). R831b is a new atypical H plasmid in that it is small (50 Md(f)100 for other H plasmids) and is not temperature sensitive for transfer. It produces pili serologically related to IncM pili (D. Bradley, personal communication). Its relationship to H1 and H2 plasmids is under test.

MINOR PROMOTERS IN THE *rplJL-rpoBC* OPERON IN *ESCHERICHIA COLI*

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The *rplJL-rpoBC* operon of *E. coli* encodes 5 components of the cell's transcription-translation machinery: ribosomal proteins L10, L7/L12 (both encoded by *rplL*), and RNA polymerase subunits *B* and *B'*. The recent application of molecular cloning techniques to this system has established that all 4 genes are co-transcribed from a common promoter in the order *rplJ-rplL-rpoB-rpoC*, and suggests that transcription of the downstream *rpoBC* genes is regulated at a site between *rplL* and *rpoB*, called *rpoU* (for Uncoupling). At least 2 internal promoters are also present, whose effective strength is far below that of the major promoter: (1) capable of serving *rplL*, *rpoB* and *rpoC*; (2) capable of serving *rpoB* and *rpoC*. Based on our results and those of other workers it appears likely that each of the genes in this operon is provided with its own promoter. We speculate that the weak internal promoters may reflect an archaic condition existing before the development of the operon organisation. In any case they may be biologically significant if they become more active under conditions differing from those so far tested.

THE TOL PLASMID FROM *PSEUDOMONAS*: CURING, EXCISION AND TRANSPOSITION

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The Tol^+ strain *P. putida* mt2 can become Tol^- by loss of the whole TOL plasmid or by excision of a 40 kb segment. However, in a series of "cured" derivatives from which no plasmid could be isolated DNA from parts of TOL was now carried chromosomally. Cleavage mapping and heteroduplex analysis of cloned fragments show that the region to be excised is bounded by a pair of directly repeated sequences of 1·4 kb. Each of these probably has a short invertedly repeated sequence at its ends. The segment of TOL suggested by others to constitute a transposon was 80 kb long and overlapped the 40 kb segment at both ends.

FINE STRUCTURE MAPPING OF THE GENES FOR THE RNA POLYMERASE SIGMA SUBUNIT (*rpoD*) AND THE GENE FOR DNA PRIMASE (*dnaG*) IN *E. COLI* AND *S. TYPHIMURIUM*

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The gene, *rpoD*, for a subunit (σ) of the transcriptase of *E. coli* and *S. typhimurium* is close to that of the RNA polymerase used in DNA replication (*dnaG*). We have elucidated the basic organisation of these genes by cloning the bacterial DNA into λ vectors. Combined restriction, deletion and functional analysis of a fragment cloned from each of these species shows that the two genes are immediate neighbours, reveals the existence of a new gene adjoining *dnaG* and indicates in which direction *rpoD* is transcribed. Our data suggest that these genes do not form a single operon.

SOME ANOMALIES IN COMPARATIVE GENE EXPRESSION IN GRAM-NEGATIVE BACTERIA

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Studies comparing regulation of gene expression between strains and organisms within Gram negative bacteria bring out two anomalous features:

(1) Genes for omnipresent functions such as tryptophan biosynthesis on transfer between the enterobacteria show normal control (in respect of both maximal level and extent of repression) in all of them, from *E. coli* to *Proteus mirabilis* (Manson and Yanofsky, *J. Bact., 126,* 679-689; 1976). Dispensable functions generally show the same behaviour; but the *Proteus* tribe represent an exception for in these anomalously low expression is found for several plasmid-borne systems, *e.g., lac,* β-lactamase and F-type piliation (see Baumberg and Dennison, *J. Bact., 123,* 278-286; 1975). *E. coli trp* genes transferred to pseudomonads and their relatives are again expressed at around maximal levels, though they are not repressed in the presence of exogenous tryptophan (see *e.g.*, Nagahari, Koshikawa and Sakaguchi, *Molec. Gen. Genet., 171,* 115-119; 1979). However, *lac* (on the transposon Tn951 inserted into RP1) again shows weak expression in *Pseudomonas* (Baumberg, Cornelis, Panagiotakopoulos and Roberts, submitted for publication), as does β-lactamase in *Rhizobium leguminosarum* (Beringer, *J. Gen. Microbiol., 84,* 188-198; 1974).

(2) *A priori* considerations, as well as a handful of published experiments, suggest that enzyme inducibility/repressibility have evolved as being of selective advantage in nutritionally

fluctuating environments. Some observations hard to reconcile with this idea are: (a) *E. coli* isolates show for the arginine biosynthetic enzymes a continuum from K12-type repressibility, through B-type near-constitutivity, to marked inducibility (S. J. Collinson, unpublished data); (b) a *Shigella dysenteriae* strain carries a *trp* mutation with both down-promoter and partial o^c effects; it readily reverts to give a more *E. coli*-like control phenotype (Miozzari and Yanofsky, *Proc. Natl. Acad. Sci., U.S.A., 75*, 5580-5584; 1978); and (c) in *Proteus mirabilis*, the arginine biosynthetic enzymes are virtually constitutive (Prozesky, *J. Gen. Microbiol., 55*, 89-102; 1969).

EVOLUTION OF MATING PREFERENCES

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Frequency-dependent mating is an inevitable outcome of sexual selection by female preference. Experiments in which females are offered a choice between males with different genotypes show that the rarer males often do gain the advantage, suggesting that preferential mating is a general mechanism of sexual selection. Fisher postulated that mating preferences will first evolve in favour of some advantageous characteristic in the males. The preferences will then give themselves an advantage, since the preferential matings produce offspring with preferred genotypes who also carry the genes for the preference: the preference increases the frequency of the preferred genotypes and hence also the frequency of the genes for the preferences. Fisher argued that this selection will proceed at a geometrically increasing rate. But his argument was not based on a genetic model and ignores the genetic mechanism of recombination by which the genes for the preference are passed to disadvantageous individuals who lack the preferred character as well as to those who possess it. The rate of selection is never as fast as Fisher suggested and depends on the genetics of the character and its preference. Computer models show that in general preferences can only evolve to high levels as a result of a "peak shift" in the females' response to the male character, such that they respond more readily to more extreme developments of the character. The mating preferences can then evolve in several stages as new alleles are selected to enhance the development of the character. But this selection may be a slow process. In monogamous species, selection can be very slow indeed and soon brought to a halt by natural selection; but in the absence of natural selection, sexual selection always produces the same ultimate outcome in both polygynous and monogamous species. These results are compatible with data on levels of sexual dimorphism in polygynous and monogamous species. The models do not allow the evolution of preferences favouring heterozygotes separately from homozygotes: preferences for heterozygotes are usually eliminated. This presents a difficulty for the theory: in natural populations of birds, assortative preferential mating of heterozygotes has been observed.

BEHAVIOURAL CORRELATES OF INFECTIOUS HEREDITY

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Six incipient species or semispecies compose the *D. paulistorum* species-complex. Among them intense reproductive isolation exists in assorted forms and with varying genetic architectures. Two types are of interest here: 1. Sexual isolation—making matings within semispecies much more likely to occur than between semi-species. This behavioural isolation is fostered by polygenes distributed all over the 3 pairs of chromosomes possessed by this superspecies; and 2. Hybrid male sterility—occurring only as a result of forced intersemispecific crosses; this thoroughgoing (even into backcrosses) sterility is related to maternal genotypes. If it is a hybrid one, sons are sterile. The *D. paulistorum* species-complex is a relatively rare but precious example of species *in statu nascendi*. In direct observation of the mating of *D. paulistorum* semi-species females with heterogamic (unlike) or homogamic (like) males, it was demonstrated that aged females' sexual selection did not significantly differ from that of young females. Previous heterogamic copulatory experience did not consistently change the degree of sexual isolation; however, females with homogamic copulatory experience showed a significantly higher preference for homogamic males. A test of the proportions of homogamic matings relative to total matings indicated significant differences between subjects with homogamic experiences and naive ones across all combinations.

CELLULAR AND CHROMOSOMAL DIFFERENTIATION IN MOUSE EMBRYO DEVELOPMENT

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Temporal and regional studies of X chromosome activity in early mouse embryos from cleavage to gastrulation will be described. These support a "stem" line model of cellular differentiation during development. In this model X chromosome differentiation (inactivation of one X in females) occurs in different populations of cells as they "depart", or "terminally differentiate", from a pluripotent stem cell line. In addition, recent studies have shown that a cycle of X chromosome inactivation and reactivation occurs in the female germ line. This reactivation, which occurs around the time XX germ cells enter meiosis, is the only example of the reversal of the non-functional state of the silent X chromosome. The implication of the model for cellular differentiation in development will be discussed.

DEVELOPMENTAL GENETICS OF THE *t*-COMPLEX

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The naturally occurring haplotypes of the *t*-complex on chromosome 17 of the mouse have been of interest in developmental genetics for many years on account of their unusual properties, including distortion of transmission ratio and suppression of recombination in a segment of chr. 17, as well as embryonic lethality and male sterility. Spontaneous "mutants", with alterations in one or more properties, arise in the laboratory, mainly by crossing-over. Study of them has shown that *t*-haplotypes involve a segment of chromosome one or more *G*-bands long and that some properties such as lethality and sterility, are due to factors located at specific points in this segment. Others, such as crossover suppression, depend on all parts of the haplotype. It is suggested that *t*-haplotypes involve a change in intercalary repetitious DNA, affecting both chiasma formation and the control of gene expression. Apparently not all structural genes in the segment of chr. 17 concerned are affected; those of which the expression is altered may be preferentially those concerned with cell surface antigens.

AN EXPERIMENTAL COMPARISON OF THE LIMITS ATTAINED BY INDIVIDUAL AND WITHIN-FAMILY SELECTION

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Under the infinitesimal model with additive gene action and independent loci, individual and within full-sib family selection should lead to the same selection limit, as in the latter the effective population size is doubled but only half of the genetic variance is used (Robertson, *Proc. Royal Soc. London, B, 153*, 234, 1960). However, when the heritability of the trait, the selection intensity and the family size are large, a higher limit is expected from within family selection, as the reduction in genetic variance is smaller than under individual selection (Dempfle, *Genet. Res., 24*, 127, 1975). Furthermore, the effective population size with individual selection is smaller than the actual size as all parents do not contribute offspring to the selected group with equal probability (Robertson, *Genet. Res., 2*, 189, 1961). To check Dempfle's theory, we carried out an experiment using individual and within full-sib family selection for low sternopleural bristle number in the Dahomey ($h^2 \sim 0{\cdot}6$) population of *D. melanogaster*. Six replicate lines per treatment were selected until a limit was attained (17 generations) and in each replicate six families of size 20 were scored per generation and the best 10 per cent selected (individual selection: 6/60 of each sex; within-family selection: the best male and female of each family).

The limits attained by both sets of lines were the same. Several factors may have contributed to lowering the probability that the within-family lines surpassed the individually selected lines. In both sets: (i) realized heritabilities were significantly smaller than base

population estimates, (ii) variances were considerably reduced with selection, (iii) variability between replicates of the response to selection decreased as the number of generations elapsed increased, and was much lower than predicted (Hill, *Biometrics, 30,* 363, 1974), (iv) actual selection intensities were smaller than expected; and in the within-family selected lines: (v) whole families were lost and their effective size thereby reduced.

THE USE OF TERATOCARCINOMAS IN DEVELOPMENTAL BIOLOGY

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The stem cells of mouse teratocarcinoma tumours have many properties in common with undifferentiated embryonic cells. Both differentiate into a variety of cell types with different biochemical, antigenic and ultrastructural properties. When teratocarcinoma cells are put into an early embryonic environment they sometimes come under the control of the host embryo and participate in normal development. These characteristics have led to the use of teratocarcinomas as a model for studying mammalian differentiation in vitro. Evaluation of cellular interactions between embryonic and teratocarcinoma cells will be important to establish the validity of this model.

THE ROLE OF THYMIDINE KINASE IN DNA REPAIR AND MUTAGENESIS IN FRIEND LEUKAEMIA CELLS

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Friend murine leukaemia cells have an abnormally high mutation rate to thymidine kinase deficiency. Deficiency of this enzyme results in increased cell killing by ultra-violet irradiation as evidenced in all of twelve thymidine kinase deficient clones tested. The increased cell killing in thymidine kinase deficient cells is reflected in increased mutagenesis (per unit dose of mutagen) to 6-thioguanine resistance. In light of these results, the hypothesis that thymidine kinase is a key enzyme in an error-free DNA repair system will be discussed.

GENETIC CONTROL OF THE RESPONSE OF TARGET ORGANS TO ANDROGENS IN THE HOUSE MOUSE, *MUS MUSCULUS*

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The genetic factors controlling tissue response to androgens are being investigated by measurements of testosterone levels and the sensitivity of target organs to exogenous androgens. Serum testosterone levels of 8 week old males from five inbred strains have been measured. Strains PHL:YH and C57B1/Fa have significantly lower levels (means 0·8 and 1·9 ng/ml respectively) than strains CBA/FaCam, PHL and PHH (means 9·3, 12·5 and 14·8 ng/ml respectively). No strain differences in the target organ weight/body weight ratio have been found in prostate, preputial or seminal vesicles of intact animals, but the kidneys of PHL:YH and C57B1/Fa are smaller with respect to body weight than those of the other three strains. Target organs' sensitivity was measured by castrating mice at four weeks of age and injecting various doses of testosterone proprionate in order to mimic the events which normally occur at puberty. The increase in weight of each organ was taken as a measure of its response to androgens.

The dose-response curves show that all the target organs of strain PHL:YH are more sensitive than those of C57B1/Fa or CBA/FaCam. The preputials and prostates of C56B1/Fa and CBA/FaCam show similar responses, whereas the seminal vesicle of C57B1/Fa is more sensitive than that of CBA/FaCam and the kidney of CBA/FaCam is more sensitive than that of C57B1/Fa. In general, the kidney is the most sensitive target organ requiring a lower

concentration of exogenous androgen than the other target organs to mimic the normal weight at puberty. The genetic factors causing strain differences in target organ sensitivity may thus be seen to have both general and tissue-specific effects on the response to testosterone. Experiments are in progress to compare these differences in target organ sensitivity with the 5-d reductase activity and androgen receptor properties of the target organs.

A FAMILIAL VARIANT OF HUMAN CHROMOSOME 9 BEARING A NUCLEOLUS ORGANISER REGION

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Chromosome analysis in a dysmorphic retarded child and several maternal relatives showed a striking polymorphism of the satellited short arm of one homologue of chromosome 22, but no obvious chromosome abnormality. It was subsequently revealed by *in situ* hybridisation with ribosomal gene probe and by silver staining, that a nucleolus organiser region was located on the distal end of the long arm of one homologue of chromosome 9 in those relatives that had the polymorphic 22. Although the proposita did not have the variant 9 chromosome, gene dosage studies revealed an approximate 50 per cent increase in activity of red cell adenylate kinase, a gene located on the distal end of the long arm of chromosome 9 (Ferguson-Smith *et al.*, *Human Genetics*, *34*, 35, 1976). These findings illustrate perhaps for the first time, how information from the human gene map can be used in the detection and interpretation of chromosome aberrations beyond the resolution of the microscope.

THYROID ANTIBODIES AND THYROID FUNCTION IN DOWN'S SYNDROME

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Thyroglobulin antibodies (TGA) and thyroid function were assessed in 24 patients with Down's syndrome, their mothers and 3 sibs, compared to 10 age matched normal controls and their mothers. Cytogenetic study was undertaken for the patients. Tanned red cells haemagglutination test was used for detection of TGA, and radio-immuno-assay for T_3, T_4, and TSH.TGA titres were above the normal level in 29·2 per cent of the patients and 45·8 per cent of their mothers, while they were very high in 20·8 per cent of the patients and 33·3 per cent of their mothers. No TGA were detected in the control group. T_3 in serum was higher than normal levels in 54·17 per cent of patients and 41·67 per cent of their mothers. T_4 and TSH were high in 12·5 per cent and 45·83 per cent respectively in patients and within normal range in their mothers. In controls, normal levels were obtained for T_3, T_4 and TSH. The results point to the possible involvement of maternal TGA in the predisposition to non-disjunction in the offspring.

THE DUFFY BLOOD GROUPS AND MALARIA IN MONKEYS

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Almost all Europeans have the red cell antigen Fy^a or Fy^b or both, controlled by alleles at the *Duffy* locus: these people also have a third antigen Fy3. The phenotype Fy(a-b-) Fy:-3, though very rare in Whites is very common in Blacks. Miller and his colleagues (*Science*, 1975, *189*, 561-562 and *N. Engl. J. Med.*, 1976, *295*, 302-304) showed that Fy(a-b-) Fy:-3 red cells, unlike cells of other Duffy phenotypes, are not invaded *in vitro* by merozoites of *Plasmodium knowlesi* or, *in vivo*, of *Plasmodium vivax*. Professor S. Cohen of Guy's Hospital Medical School wondered whether monkeys showed the same correlation between the presence of Duffy antigens and susceptibility to invasion. He provided blood from 7 Old World monkeys (4 rhesus and 3 kra) which are susceptible to *P. knowlesi* but not to *P. vivax* and 3 New World

monkeys (douroucouli) which are susceptible to both. None of the monkeys has Fy^a or Fy^b. The rhesus and kra have some Fy3 antigen, but less than man, while the douroucouli have very little Fy3. In monkeys, therefore, Fy^a and Fy^b are not the receptors for invading merozoites. If Fy3 were the receptor, the results do not fit for *P. vivax.*, since rhesus and kra cells are not invaded yet have more Fy3 than those of douroucouli which are invaded; the results are in accord for *P. knowlesi* where the presence of Fy3, in smaller amounts than in man, matches the ability of merozoites to invade red cells. Of interest from the blood group point of view, the monkey work also proved that Fy3 can be present, albeit relatively weakly, in the absence of Fy^a and Fy^b.

THE INHERITANCE OF CORTICOSTERONE SYNTHETIC CAPACITY MEASURED IN ISOLATED MOUSE ADRENAL CELLS

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Using a suitably modified adrenal dispersion technique, originally described for rat adrenals (Barofsky *et al.*, *Exp. Cell Res.*, *79*, 263, 1973) strain specific differences in maximal ACTH response were observed between cells prepared from adrenal pools from DBA/2J, CEA/Cam and C57BL/Tb male mice. F1 hybrid strains, formed by crosses between C57 and CBA mothers and DBA fathers, possessed CBA dominant and intermediate phenotypes, respectively. Temporal changes in steroid production were examined in DBA, C57 and the (DBA♀×C57♂) F1 and the results compared with those of a previous study which used adrenal slices (Doering *et al.*, *Biochem. Genet.*, *8*, 101, 1973). When individual adrenal pairs were dispersed separately and assessed for their synthetic capacities, parental strains, and both reciprocal F1's of DBA×C57, displayed discontinuous distributions suggestive of different functional states. The F1 (DBA♀×C57♂) displayed higher mean and variance than the F1 (C57♀×DBA♂). Backcrosses of these F1's to both parental types demonstrated greater variances than either pure line which could be attributed to a great number of phenotypic classes. This evidence strongly suggested genetic segregation which was likely to be related to only a few gene differences. Any backcross using the F1 (DBA♀×C57♂) as a mother was observed to be suppressed relative to the equivalent backcross from the higher, more variable reciprocal F1. This effect was thought to consist largely of differences in maternal effects on the developing foetal adrenal/pituitary axis which has been noted already in a non-genetic context (Milkovic *et al.*, *Endokrinologie*, *68*, 60, 1976).

GENETIC HYPOTHYROIDISM IN *PETITE* MICE

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A new autosomal recessive petite (*pet*) has been recovered in RS/J strain mice that causes an ateliotic dwarfism recognisable by three weeks of age. Dietary supplementation with thyroid powder repaired growth and sterility and led to studies of thyroid morphology and function. Mutant mouse thyroid glands are atrophic and individual follicles are reduced in number and size, but contain some colloid. Serum T3, T4 and free T4 level are very low or undetectable. Incorporation of radio-iodine by *pet/pet* thyroid is reduced 85-90 per cent with respect to normal litter-mates. Serum TSH levels are significantly elevated in *pet/pet* mice and injection of TSH did not improve thyroidal radio-iodine uptake. Thus the mutant gene acts within the thyroid gland to block normal responses to TSH. The resultant hypothyroidism has numerous systematic consequences, including depressed pituitary hormone storage, nervous system defects, adrenal disturbances, growth failure, and infertility.