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MUTATIONAL EVOLUTION OF AN ARCHAEBACTERIAL GENE

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Recently Dunn *et al.* (*Proc. Natl. Acad. Sci., 78*, 6744, 1981) published the complete nucleotide sequence and codon usage pattern for the bacteriorhodopsin precursor gene of *Halobacterium halobium*. This DNA sequence is particularly interesting because it is both the first for a GC-rich bacterial gene, and also the first for an archaebacterial species. Archaebacteria have been shown to differ profoundly, in aspects of cell wall chemistry, lipids, transcription, translation, and 16S RNA sequences, from the eubacteria (Woese, *Zbl. Bakt. Hyg. I Abt. Orig. C, 3*, 1, (1982). The *H. halobium* sequence has, therefore, been examined for evidence of its evolution and mutability.

The following methods have been used. (1) Codon usages expressed in terms of AT-rich, GC-rich, neutral, and unique codons (Clarke, *J. theoret. Biol., 96*, 461, 1982). (2) Sites of potential premature chain-termination mutations arising as a result of transitions or transversions. (3) The proportion of RNY-type codons (Shepherd, *J. Molec. Evol., 17* 94, 1981) in the correct, and incorrect, reading frames. (4) Codons arising from RNY runs by transversions or frameshifts. (5) Location of small internal duplications. (6) Identification of potential *dam* and *mec* sites. (7) Potential frameshift mutation hot-spots in runs of repeated or reiterated bases (Clarke and Miller, *J. theoret. Biol., 96*, 367, 1982).

PAPILIO "NANDINA" AND THE EVOLUTION OF MINICRY

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We have shown (Clarke, C. A., *Systematic Entomology, 5*, 49, 1980) that *Papilio* "nandina" is a hybrid between two East African Swallowtail butterflies, one, *Papilio dardanus*, mimetic and the other, *Papilio phorcas*, non-mimetic. Both are polymorphic in the female. In the wild only male hybrids have been found, but in captivity we have also produced many females of several forms. Interestingly, the mimetic pattern of *P. dardanus* is very little broken down in the female hybrids, and this seems to contradict the view (based on race crosses in *P. dardanus*) that mimicry is the result of a major mutation followed by the accumulation of modifying genes which perfect the pattern (Clarke, C. A. and Sheppard, P. M., *Heredity, 14*, 163, 1960). Possible reasons for our "nandina" findings will be discussed.

P. "nandina" is also of interest from the point of view of the dominance of the female forms in *P. phorcas*. On the Bernard–Vane–Wright hypothesis that male-like is the "phase finale" (Bernardi, G., *Proc. Specialised Symposia, XVIth Int. Cong. Zool.* (Washington), *4*, 161, 1963), (Vane-Wright, R. I., *Symp. Roy. Ent. Soc. Lond., 9*, 56, 1978), and not the primitive form, one would expect that male-like (green) would be dominant to non-male-like (yellow) but the reverse is the case.

ON REAL AND IMAGINARY PROBLEMS OF MOLECULAR MODELS OF SPECIATION

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Several recent molecular (genomic) models of speciation have elicited several recent attempts at criticism. The strengths and weaknesses of such models and the real and imaginary problems posed by the criticism will be assessed. An argument will be developed that molecular models, based primarily on DNA sequence turnover in nuclear genomes (for review see Dover, 1982, *Nature*, *299*, 111–117), cannot be forced into traditional cul-de-sacs associated with "chromosomal", "single locus" or "multi-loci" models based on classical concepts of selection and drift. Rather the strengths of the models (the potential to distribute mutants in populations, in unexpected cohesive patterns) and their weaknesses (reflecting current ignorance of molecular biology concerning the relationship between genotype and phenotype) will be discussed, with examples.

THE EVOLUTION-RELATED PARAMETERS OF MEIOTIC GENE CONVERSION IN VARIOUS FUNGI AND *DROSPHILA*

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The evolutionarily important parameters of meiotic gene conversion were described by Lamb and Helmi (*Genetical Research*, *39*, 199–217, 1982) as *c*, the conversion frequency (as a fraction); *b*, the frequency of a particular allele of a pair in meiotic tetrads or octads with aberrant segregation ratios, and *d*, the disparity in direction of gene conversion. Suitable data have been analysed from *Ascobolus immersus*, *Drosophila melanogaster*, *Saccharomyces cerevisiae*, *Sordaria brevicollis* and *Sordaria fimicola*.

c varied from zero to over 0·3; different loci often had very different average *c* values, presumably reflecting different frequencies of hybrid-DNA. Alleles at the same locus typically showed 1·5 to 12-fold variation in *c* values, with an irregular scatter, not a "normal distribution". Loci often showed some *b* values near the expected minimum (0·25, all conversion to mutant) and maximum (0·75, all conversion to wild-type) values, so disparity in conversion direction is often extreme, as well as frequent. *b* values for a single locus did not show a "normal distribution": there was random scatter or bi- or tri-modal distributions, with peaks at extremes as well as centrally. Overall, disparity to wild-type was as common as disparity to mutant, but some loci had a clear excess of disparity in one direction. Most loci had no significant correlation between *b* and *c* values, but significant correlations (positive and negative) occurred more often than expected by chance.

y, the force of meiotic gene conversion in evolution, is $c\ (b-0{\cdot}5)$. Graphs relating equilibrium allele frequencies to *y*, under various conditions of selection, mutation and dominance, showed that gene conversion is an important factor in evolution.

A GENETIC MODEL FOR THE *Ac* LOCUS WHICH CONTROLS CYANOGENIC GLUCOSIDE PRODUCTION IN WHITE CLOVER (*TRIFOLIUM REPENS L.*)

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Many European populations of white clover exhibit polymorphism at the locus *Ac*/*ac* which controls the production of two cyanogenic glucosides, linamarin and lotaustralin. Plants possessing the allele *Ac* produce both glucosides. Plants homozygous for the null allele *ac* do not produce either. Heterozygotes (*Ac ac*) produce less than half the amount of glucoside than their homozygous (*Ac Ac*) parents (Hughes and Stirling, *Euphytica*, *31*, in press, 1982). There are apparently several *Ac* alleles which control the production of different amounts

of the glucosides (Hughes, pp. 495–508 in *Cyanide in Biology*, ed. B. Vennesland *et al.*, 1981, Academic Press). Plants possessing different *Ac* alleles show variation in the amounts of cyanogenic glucoside that they produce at different temperatures of growth and in the quantities of linamarin and lotaustralin synthesized *in vivo* from their precursor amino acids, L-valine and L-isoleucine (Collinge and Hughes, *J. exp. Bot.*, *35*, 154–161, 1982). A particulate (microsomal) fraction from plants of genotype *Ac Ac* and *Ac ac* will convert both amino acids, oxime and nitrile intermediates into the immediate, cyanohydrin precursors of the respective glucosides in the presence of NADPH. Similar fractions from plants of genotype *ac ac* will not convert any of the precursors to the cyanohydrins. Studies on the *in vitro* biosynthesis of the glucosides show that one set of enzymes is responsible for the production of both glucosides. At least two steps in the biosynthetic pathway of the cyanohydrins are blocked in plants of genotype *ac ac* (Collinge and Hughes, *Arch. Biochem. Biophys.*, 217, in press, 1982 and unpublished results). Results obtained from *in vivo* feeding experiments suggest that the last enzyme, which glucosylates the cyanohydrin of linamarin is missing in plants which do not produce either glucoside. This represents a third blockage in the pathway (Hughes and Conn, *Phytochemistry*, *15*, 697–701, 1976). The particulate fraction carrying the biosynthetic enzymes has been purified using Percoll density gradient centrifugation, and Sephacryl molecular exclusion chromatography to assist in determining whether there are polypeptide differences associated with the locus which may be detected by SDS-PAGE. A model for the *Ac* locus is discussed in the light of these new results.

BIOCHEMICAL CHARACTERISATION OF THE *Li* LOCUS IN WHITE CLOVER (*TRIFOLIUM REPENS L.*)

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The locus, *Li*, controls the presence or absence of linamarase (β-glucosidase E.C.3.2.1.21) activity in white clover leaves. If linamarase activity is assayed using the natural substrate, linamarin, plants homozygous for functional alleles (*Li Li*) show high activity, heterozygous plants (*Li li*) show intermediate levels of activity and plants homozygous for the nonfunctional allele (*li li*) show no measurable activity (Maher and Hughes, *Biochem. Genet.*, *8*, 13, 1973). Linamarase can be purified to a simple SDS-PAGE polypeptide (62 K daltons) by a combination of molecular exclusion and ion exchange chromatography. The enzyme represents about 6 per cent of the total soluble protein extracted from *Li Li* leaf tissue and is the major glycoprotein exchanging with α-methyl *D*-mannoside on concanavalin A. Immunological tests show that *li li* plants do not contain an enzymically inactive protein and this is confirmed by SDS-PAGE of crude leaf extracts and mannosylglyco-proteins, where linamarase can be seen as a distinct band in *Li Li* and *Li li* extracts which is absent or very reduced in *li li* extracts (Hughes and Dunn, *Plant Mol. Biol.*, in press). The relationship of linamarase to other white clover β-glucosidases is discussed.

INCREASED SENSITIVITY TO THE INDUCTION OF CYTOGENETIC ABERRATIONS BY MITOMYCIN C IN THYMIDINE KINASE DEFICIENT FRIEND LEUKAEMIA CELLS

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It has previously been reported that deficiency of the enzyme thymidine kinase results in increased sensitivity to cell killing and mutagenesis by ultra-violet irradiation (UV) (McKenna, P. G. and Hickey, I., *Cell Biology International Reports*, *5*, 555 1981) and a variety of chemical mutagens (McKenna, P. G. and Yasseen, A. A., *Genetical Research*, in press). This has been interpreted by McKenna and colleagues to indicate that thymidine kinase has a significant part to play in ensuring accurate DNA repair after mutagen damage.

Further experiments have been carried out to ascertain if deficiency of the enzyme also results in an increased frequency of cytogenetic aberrations after treatment with mitomycin C (MMC). The frequency of 13 types of cytogenetic aberrations, including chromosome- and

chromatid-types were assayed after treatment with MMC in wild-type clone 707 Friend cells and in the thymidine kinase deficient subclone, 707BUF. Two doses of MMC were used and cells were harvested at 15, 29 and 43 hours after treatment. There was a significantly higher frequency of all types of aberrations in the thymidine kinase deficient subclone relative to wild-type cells following both doses of MMC. Furthermore, cytogenetic aberrations persisted longer in subclone 707BUF.

These results give further support to the theory that thymidine kinase plays a key role in ensuring accurate DNA repair.

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NEGATIVE COMPLEMENTATION OF THE *E. COLI RECA*$^+$ GENE

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In DNA-damaged *E. coli* recombination activity relying on the *recA*$^+$ protein can be inhibited by plasmids encoding the *recA* control sequence and between 22 and 79 per cent of the contiguous structural gene. Gene derepression using *recA*$^+$ protease action is, however, little affected. A discrete minimal size of amino-terminal truncated *recA* protein was needed to inhibit recombination. Plasmids encoding amino-terminal fragments smaller than 22 per cent, or a carboxy-terminal fragment of *recA* protein, had no effects on recombination. It is proposed that full sized and truncated protein subunits interact in an amino-terminal domain to generate multimers unable to catalyse recombination. The notion that *recA*$^+$ protein has more than one physical domain is also suggested by its biphasic loss of circular dichroism during denaturation.

Negative complementation of recombination can be used to determine whether DNA-damage induced responses use *recA* recombinase or protease activities. UV mutagenesis of *E. coli* WP2 was not reduced by inhibiting recombination repair, even in bacteria additionally deficient in excision repair. Similar induced reactivation of λ could be eliminated, without reducing induced clear-plaque mutagenesis. Thus, inducible responses promoting survival of both *E. coli* and λ use increased levels of *recA* recombinase activity. Mutagenesis, however, is not an intrinsic part of these inducible responses. Rather it can be a separate and rare event which contributes little to the overall survival of a DNA-damaged population.

GENETIC EVIDENCE FOR THE EXISTENCE OF A PSEUDOGENE RELATED TO A MAJOR REGULATORY GENE IN *ASPERGILLUS NIDULANS*

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Arst and Cove (*Molec. gen. Genet., 126*, 111, 1973) showed that loss of function mutations (designated *areA*^{r}) in *areA*, a positive acting regulatory gene mediating nitrogen metabolite repression in *Aspergillus nidulans*, lead to inability to utilise nitrogen sources other than ammonium. This work establishes the existence of a gene designated *areB*, on the left arm of linkage group I, where mutations can suppress *areA*^{r} mutations in a locus-specific manner for expression of apparently all of the genes under *areA* control. *areB* mutations are partially dominant in diploids, extremely rare and, to varying degrees, deleterious. In agreement with their ability to suppress *areA*^{r} mutations, *areB* mutations lead to nitrogen metabolite partially depressed enzyme levels. All five *areB* mutations selected are associated with chromosomal aberrations, translocations in four cases and probably a short paracentric inversion in the fifth. Two of the translocations, although induced in different strains in separate experiments, have almost identical breakpoints in both linkage groups I and VII. This suggests that chromosomal rearrangements of a highly specific nature can give rise to an *areB* mutant phenotype. We propose that *areB* might be an *areA*-related pseudogene able to be activated by chromosomal reaarangements resulting, for example, in fusion to an active promoter.

AN INTRON IN THE *AM* (GLUTAMATE DEHYDROGENASE) GENE OF *NEUROSPORA CRASSA*

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The *N. crassa am* gene has been cloned and partially sequenced. The sequenced portion includes the 55 non-coding bases 5′ to the initiation codon, the first 99 codons, and a 67 base intervening sequence interrupting codon 15. The presence of the intron may explain the surprisingly high recombination frequency that had been found between point mutations in codons 5 and 25. The intron splicing junctions conform to the eukaryote consensus. A less usual feature is the presence of 8 base-pair inverted repeats just inside the splicing junctions. The 5′ non-coding sequence includes the CACCAC motif observed in several other yeast and Neurospora genes (possibly to do with ribosome binding) but not other features of note. Codon usage is highly biased in a manner similar to that seen in several other Neurospora genes and quite different from that seen in yeast. Experiments on DNA-mediated transformation (performed in collaboration with J. A. Kinsey of the University of Kansas) have shown that the *am* gene can be integrated and expressed (though not usually at normal level) at unusual sites in the genome.

SUPPRESSIBLE MUTATIONS AT THE *NIAD* LOCUS HAVE THE PROPERTIES ATTRIBUTABLE TO CHAIN TERMINATION MUTATIONS

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Three cosuppressible mutations have been isolated previously, *alX*4, *sB*43 and *alcA*125 (Roberts *et al. Molec. gen. Genet., 177*, 57–64, 1979). These mutations are suppressed by informational suppressors from at least four loci. The three suppressible loci constitute the unleaky class of mutation, and all of them are assumed to be point mutations since they are revertible. A suppressible mutation within the *niaD* gene was sought because mutants in nitrate reductase can be assayed for enzyme activity and cross reacting material, and inter-allelic complementation has been observed within this gene, providing simple criteria for identifying putative nonsense mutations.

Mutants in *niaD* were selected after photodynamic or diethyl sulphate mutagenesis. Two of the mutants were corevertible with *alX*4 and these were unleaky, revertible, non complementing and phenotypically suppressible by aminoglycoside antibiotics (Martinelli and Roberts, this meeting). Neither of the mutants had any activity *in vitro* and showed no cross reacting termination mutations and suggest that the informational suppressors can act upon chain termination codons.

DIAGNOSIS OF NONSENSE MUTATIONS IN *ASPERGILLUS NIDULANS* BY MEANS OF PHENOTYPIC SUPPRESSION

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Alleles *alX*4, *sB*43, *alcA*125, and *niaD*500 which are genotypically suppressible are also suppressed phenotypically by one or more aminoglycoside antibiotics, and the converse is true for other alleles at these loci. Other known and suspected missense mutations are not suppressed by either method. The properties of the suppressible alleles are consistent with their being nonsense mutations (Roberts *et al. Molec. gen. Genet., 177*, 57–64, 1979). Phenotypic suppression could therefore be used to distinguish nonsense from missense mutations.

Paromomycin suppresses all those alleles which are suppressible and has the strongest activity. Tobramycin is similar. Neomycin, gentamycin and sisomycin are weaker. Hygromycin and G418 suppress only *alX*4 well. Kanamycin, apramycin and netilmycin are poor. Paromomycin or tobramycin are recommended for screening unknown mutants.

AN INFORMATIONAL SUPPRESSOR IN *ASPERGILLUS NIDULANS* HAS AN ALTERED RIBOSOMAL PROTEIN

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The *suaC*109 mutation was isolated as a suppressor of the putative nonsense mutations *alX*4 and *sB*43 and also acts on *alcA*125 and *niaD*500 (Roberts *et al. Molec. gen. Genet.*, *177*, 57–64, 1979). It does not suppress known missense mutations at *yA* and *gdhA* nor other alleles of *alX*, *sB*, *alcA*, or *niaD*. The mutation, which is recessive, also confers slow growth, altered morphology , cold sensitivity and hygromycin sensitivity, thus it was thought to be a rather special kind of ribosomal ambiguity mutant, acting only on nonsense codons.

Ribosomal proteins have been extracted from *suaC*109 and *suaC*$^+$ strains and compared. In two dimensional electrophoresis, no differences in profile were apparent. Separation of proteins on a cation exchange column shows a tenfold increase in one protein of *suaC*109. It is thus likely that *suaC*109 is a ribosomal protein mutant.

WHAT IS THE ALTERATION IN A HISTIDINOL UTILISING MUTANT OF *ASPERGILLUS NIDULANS*?

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Wild type and histidine requiring strains cannot use histidinol as a source of nitrogen or histidine. A rare histidinol utilising mutant *holA*1 was isolated after mutagenesis of a double mutant strain *hisB*147, *hisC*131. *holA*1 is 2 centimorgans from *hisC*131 on Linkage Group VIII. It acts as a physiological suppressor of all *hisA*, *hisB*, *hisC* and *hisD* and *hisEI* mutations tested to date and is probably general in effect.

The *holA*1 mutation might alter permeases as in *N. crassa* (Ho Coy Choke, *Genetics*, *62*, 725–733, 1969), or histidine regulatory proteins or proteins concerned with nitrogen metabolism. However, *holA*1 does not have elevated levels of histidine biosynthetic enzymes nor altered uptake of histidine or other amino acids. In growth tests on solid medium, histidinol does not compete for uptake with histidine into a *hisA* strain with or without the *holA*1 mutation. The requirement for histidine is saturated at a much lower external concentration by histidine than histidinol.

If the analogue amitrol is used to block histidine synthesis in *his*$^+$*hol*$^+$ strains, histidinol supports growth which is 20 per cent of normal, rate limited by some factor other than histidinol concentration. *holA*1 strains either auxotrophic for histidine, or amitrol treated, can grow at the maximum growth rate but more histidinol is required by the amitrol treated strain than the auxotroph to overcome the block.

These results are consistent with either (a) a permease system which binds but is normally unable to transport histidinol or (b) a histidinol "utilase" system, which is not histidinol dehydrogenase itself, inside the cell but normally with a very low capacity. Thus *holA*1 remains a mystery.

CLONING OF THE *PYR*-4 GENE OF NEUROSPORA

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The *pyr-4* gene of *Neurospora crassa* encodes the enzyme orotidine-5′-monophosphate carboxylyase, and is the equivalent to *pyrF* in *Escerichia coli*. In an attempt to clone the

Neurospora gene, ampicillin-resistant pyrimidine-independent colonies were selected in an ampicillin-sensitive pyrimidine auxotrophic strain of *E. coli* after transformation with a Sau 3A partial digest gene back in plasmid pRK9. The pRK9 plasmid is a derivative of pBR322, and carries ampicillin resistance. Ampicillin-resistant pyrimidine-independent transformants were obtained by this method, and two were used for further characterisation. It was fortunate that the *pyr-4* gene is one of a minority of Neurospora genes which can complement their bacterial equivalents. As a first step, Southern analysis was used to verify the presence within each transformant clone of an insert of Neurospora DNA. From these a restriction map of the *pyr-4* region was constructed, and the actual coding region localised to a 3·5 kb segment. Further studies are in progress.

CYCLOHEXIMIDE AND EMETINE RESISTANCE IN *SCHIZOSACCHAROMYCES POMBE*

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It has been suggested that cycloheximide and emetine contain the same active structure (Grollman, *Proc. Nat. Acad. Sci. USA, 56,* 1867, 1966). However, wild type cells of *Schizosaccharomyces pombe* cannot grow in presence of 60 μg/ml cycloheximide, but they can grow in presence of 200 μg/ml emetine. Cycloheximide resistant strain, *cyh1*-C7, showed better growth on yeast extract medium with 200 μg/ml emetine as compared with the growth of wild type strain. *cyh1*-C7 strain possessed ribosomes which were more resistant to cycloheximide than the wild type ribisomes (Berry, Ibrahim and Coddington, *Molec. gen. Genet., 167,* 217–225, 1978). Subinhibitory concentrations of ethanol (2·5 per cent and 5 per cent) were found to enhance cycloheximide and emetine activities against *S. pombe* strains.

STUDIES ON THE CHROMOSOMES OF HUMAN SPERMATOZOA

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Chromosome aberration plays a very important role in human mortality and morbidity and it is obviously of fundamental importance that a clear understanding of its causes and consequences is developed. At the present time, much of the information is speculative, being inferred from population studies of newborns and abortuses and it would be of considerable value if a reliable technique were available for the direct analysis of the chromosomes of human gametes. Rudak *et al.* (*Nature, 274,* 911–913, 1978) have described a technique for the visualization of the chromosomes of human sperm which relies on the *in vitro* penetration of zona pellucida-free eggs from hamsters. In this communication, studies on sperm chromosomes using modifications of this technique will be described. A variety of physiological parameters has been found to affect the efficiency of sperm penetration and chromosome visualization.

INTERGENERIC PLASMID TRANSFER IN YEASTS BY PROTOPLAST FUSION-GENETIC TRANSFUSION

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It has been found that one of the principal consequences of protoplast fusion in the yeast *Saccharomyces cerevisiae* is the transfer of extrachromosomal genetic determinants or organelles ("Genetic Transfusion") between parental nuclei with the consequent production of heteroplasmons.

The intergeneric transfer of recombinant plasmids has been demonstrated using a yeast episomal plasmid, pDB248(x), which transforms *S. cerevisiae* and *Schizosaccharomyces pombe* at high frequencies. The transfer of pDB248(x) between these two yeasts has been achieved

by protoplast fusion. This transfusion procedure is very efficient with at least one per cent of regenerated protoplasts of *S. pombe* acquiring the plasmid and provides an effective method for isolating transformants derived from transitory hybrids of the budding and fission yeasts used. The transformants obtained were found to have the characteristic rod-shape and the nuclear genotype of the fission yeast. The presence of pDB248(x) was confirmed by (i) complementation of the β-isopropylmalate dehydrogenase deficiency (*leu 1*) of *S. pombe* by the *S. cerevisiae LEU 2* gene component of pDB246(x); (ii) expression of the bacterial β-lactamase gene on pDB248(x), and (iii) high frequency reversion to *Leu* after relaxed growth. Analysis of the transformants (or *transfusants*) is in progress to ascertain whether other genetic determinants accompany plasmid transfusion.

OF BUTTERFLIES AND JERKS: IS THERE A "SECOND PROCESS" IN EVOLUTION?

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The Gould-Eldredge-Stanley theory of evolution by jerks (punctuated equilibria) maintains that neoDarwinian theory is inadequate, rather than wrong, as an explanation of evolution writ large. The central dogma of the new theory is that the species is to be seen as a basic evolutionary unit, and that organic diversity is not, as is supposed by traditional Darwinists, a simple and direct outcome of natural selection and adaptation. Rather, *speciation* is thought to generate organic diversity, and *species selection* to direct evolutionary trends, in a dialectic that occurs at a hierarchical level of organisation so far not properly considered in evolutionary theory.

Studies of adaptive radiation during race and species formation in mimetic butterflies of the genus *Heliconius* suggest, on the contrary, that diveristy does arise by individual natural selection, and is not in itself generated by, or even at the time of, speciational events. What is more, conventional gene substitutions in the butterflies produce an excellent simulacrum of punctuational events! On the other hand, the debate between macro- and micro-evolutionists has become something of a *folie à deux*, on account of the traditional resistance offered by most Darwinists to the incorporation of non-adaptive processes into evolutionary theory. The macro-evolutionists are saying something important which neoDarwinians find it hard to perceive.

HOW IS GENETIC CHANGE RELATED TO SPECIATION?

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Many models of evolution assume that there is a direct relationship between speciation and genetic change, so that patterns of genetic divergence among taxa will give information on patterns of phylogenetic relationship. Although this is often true, many exceptions are already known. Sibling species of *Drosophila*, for example, often show reproductive isolation with no detectable change in morphology, chromosome structure, or enzyme polymorphism. Here I report patterns of change at loci determining shell and enzyme polymorphism in two sibling species of *Cepaea* snails. These share many of the same polymorphisms.

The response of some of the shell polymorphisms to climatic selection is greatly influenced by the genetic background of the species in which they are found. They hence act in some respects as a "marker" for speciation. At other loci, however, response to climatic selection is apparently independent of the speciation process. At some enzyme loci also, there is far more genetic change within each species than there is between the two although other enzyme loci show consistent differences between the species.

In this case the interpretation of "evolutionary clocks" based on single loci would depend crucially on the sample of loci examined, and might produce very misleading results.

GENE FLOW AND SPECIATION

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Species are widely regarded as lineages which are isolated from each other by genetic barriers to gene flow. However, another view of the species has formed a persistent theme in evolutionary biology: it is that a species possesses a set of coadapted alleles which is stable towards the introduction of genes from other taxa. The distinction between these views leads to two questions: first, how much do incompatibilities between populations reduce the exchange of genes between them, and second, to what extent does gene flow impede the establishment of new incompatibilities? Although the hybrid zones which form at the interface between divergent populations usually involve differentiation at many loci, theory suggests that they are unlikely to present a barrier to gene flow significantly stronger than simple physical distance; neutral alleles may be somewhat impeded, but advantageous alleles will rapidly penetrate a localised barrier.

However, the answer to the second question depends on the effect of gene flow on the establishment and spread of interacting combinations of alleles, rather than on the behaviour of single loci. Gene flow may be strong enough to prevent initial divergence driven by the sporadic action of random drift, but it is unlikely to interfere with the sustained pressure of selection. An equilibrium, once established in a local population, will spread through extinction and recolonisation, rather than through the steady diffusion of genes. These considerations suggest that although gene flow forms the basis of the biological species concept, it may not play any important role in the origin of species themselves.

THE ORIGIN OF ENZYME SPECIES BY NATURAL SELECTION

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Most evolutionary theories presuppose a primitive unicellular ancestor. While present-day cells contain 10^3 to 10^4 highly specific and efficient enzymes, the primitive ancestor must have contained a much smaller number of inefficient catalysts of broad specificity. It will be shown that selection for replication rate inevitably leads to proliferation of enzyme types and increases in catalytic rates.

DOES THE MOLECULAR CLOCK KEEP TIME?

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Estimates of evolutionary rates (from macromolecular sequences) are usually made making assumptions known to be false. These include: 1, the date of divergence for two taxa is accurately known from the paleontological record; 2, all sites in the macromolecular sequence are equally subject to change; 3, every type of possible change at a macromolecular site is equally likely. It will be shown that no current method of estimating the total amount of change accounts for all the significant bias that is clearly present in mitochondrial DNA. The problem could be general. Other potential biases remaining in the sequences will be discussed.

CHROMOSOMAL REARRANGEMENTS IN SPECIATION

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Closely related species usually differ by one or more major chromosomal rearrangements, such as inversions, fusions, fissions, or reciprocal translocations. Such rearrangements can contribute to postmating reproductive isolation between populations through the production of aneuploid gametes from hybrids which are structurally heterozygous. In most higher animals and plants, aneuploidy leads to reduced viability; major duplications and deletions are usually lethal in early zygotic stages, although some amount of aneuploidy can be tolerated, particularly in plants and invertebrates.

In a variety of higher organisms major chromosomal rearrangements occur spontaneously at rates on the order of 10^{-3} per gamete per generation, and the spontaneous rate of very small rearrangements, which can not be detected cytologically, may be much higher. In outbreeding species the types of major, cytologically detectable rearrangements most commonly fixed are short inversions and centric fusions (and fissions) that entail a selective disadvantage of only a few percent in heterozygotes. Rearrangements that produce substantial heterozygote disadvantage, such as most large reciprocal translocations, or tandem fusions, are rarely fixed in outbreeding species. Partial inbreeding (e.g., self-fertilization) and reproductive compensation (for early zygotic deaths in species with extensive parental investment in offspring) effectively reduce the selective disadvantage of chromosomal heterozygotes and enhance the rate of chromosomal evolution.

Alternative mechanisms for the fixation of major chromosomal rearrangements include (1) selective advantage due to linkage or position effects, (2) random genetic drift with local extinction and colonization in geographically subdivided populations, and (3) meiotic drive. Chromosomal differences between closely related species may be important causal mechanisms promoting reproductive isolation, or could be incidental to other mechanisms of speciation.

MORPHOGENESIS AND SPECIATION IN PLANTS

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Plants and animals differ fundamentally in their processes of morphogenesis and development. The consequence is that many differences in morphological structure and shape as well as architectural arrangement and orientation in plants are often discontinuous, having discrete alternative expressions with no intermediate conditions. Many such character differences within and between plant species have been analyzed genetically and a large proportion of them appear to be governed by one or two gene loci. In contrast, most morphological characters in animals show continuous variation and their inheritance is best described by the multiple factor hypothesis. Characters that show discrete differences in animals usually are those of colour and pattern rather than structure or shape.

I propose that the prevalence of discontinuous morphological characters in plants, often under simple genetic control, is a direct consequence of the open, less integrative pattern of plant morphogenesis which can tolerate genetic changes that have relatively large morphological effects. Whereas in animals, genetic changes that can be accommodated probably have smaller phenotypic consequences and, even then, often many additional gene changes are required to modify or buffer disharmonious pleiotropic effects. Thus I suggest that fewer genetic changes may lead to large differences in morphological structures in plants than in animals. If this is true, then plant species may be more similar genetically to each other than is the case with animal species. It is also that speciation in plants will often be rapid and abrupt rather than slow and gradual and depend more on ecological circumstance than genetic revolution.