EVOLUTIONARY IMPORTANCE OF GENE REGULATION*

(gene rearrangement, structural genes, bacteria, vertebrates, evolutionary rates)

ALLAN C. WILSON

Department of Biochemistry
University of California
Berkeley, California 94720

SUMMARY

To assess the relative importance of regulatory mutations and structural gene mutations in adaptive evolution at the organismal level, two kinds of evidence were considered: 1) Evidence concerning the mechanism by which bacterial populations acquire genetically the ability to utilise a novel carbon source. (2) Evidence from studies of rates of evolutionary change in structural genes, number of chromosomes, number of chromosomal arms, hybrid inviability and anatomy of vertebrates. It is concluded tentatively that evolution at the organismal level depends chiefly on regulatory mutations and that gene rearrangement is an important mechanism for achieving altered patterns of gene regulation.

INTRODUCTION

Much attention was given in the last decade and at this symposium to a controversy concerning the mechanism of protein evolution. The central problem of evolutionary genetics, according to many research workers, has been whether sequence changes in proteins result mainly from adaptively neutral mutations. The judgement that this is a central question stems, however, from an untested assumption about the molecular basis of biological evolution. This assumption is that evolution of organisms is based predominantly on mutations in the genes coding for proteins, i.e. structural genes.

Another possibility, however, is that regulatory mutations play the major part in adaptive evolution. This article tries

* This paper is dedicated to Professor E. C. C. Lin and his students who published in 1964 a classic article demonstrating the evolutionary importance of gene regulation.

to assess the relative importance of these two types of genetic change. Although too few data are available to permit definitive conclusions, it seems likely that evolution at the organismal level depends predominantly on regulatory mutations. Structural gene mutations may have a secondary role in organismal evolution.

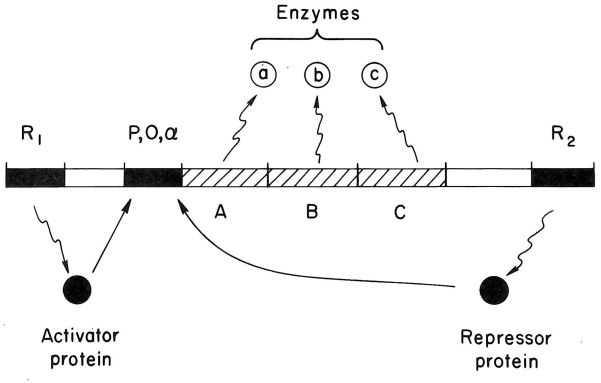


Figure 1. Types of regulatory gene. This diagram is based on studies with bacteria and shows part of a chromosome containing five regulatory loci R_1, R_2, p, o and α (solid black bars). These genes regulate the expression of loci A, B and C (hatched bars), which code for a group of three enzymes (a, b and c). R_1 and R_2 code for activator or repressor proteins that bind to promoter (p) or operator (o) sites. Mutations in any of these four types of regulatory genes or in the recently described attenuator (α) locus can affect the rate of synthesis of the enzymes coded by genes A, B and C without affecting the amino acid sequences of those enzymes (BERTRAND *et al.* 1975).

REGULATORY MUTATIONS

Regulatory mutations influence the expression of genes coding for certain proteins without necessarily affecting the structure of those proteins. Two types of regulatory mutations may be considered. First, mutations can occur in regulatory genes (Figure 1). Such mutations can affect dramatically the production though not the amino acid sequences of specific groups of enzymes. Second, the order of genes on chromosomes may change owing to inversion, translocation, duplication or deletion of genes as well as fusion or fission of chromosomes. As indicated in Figure 2, these events can bring genes into new relationships with one another. Altered patterns of gene expression sometimes result (BAHN 1971, WALLACE and KASS 1974).

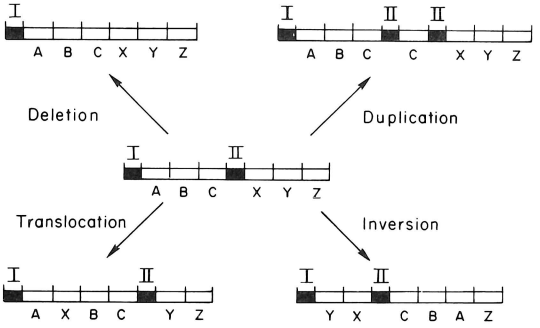


Figure 2. Types of gene rearrangement. This diagram shows how rearrangement events can bring structural genes under the influence of other regulatory loci. In the wild-type chromosome (center of diagram), one group of loci, designated I, regulates expression of genes A, B and C, while another group, designated II, regulates genes X, Y and Z. Deletion of II brings X, Y and Z under the control of I. Duplication of the C II region brings a C gene under the control of II. Translocation of X brings it under the control of I. Inversion of ABC II XY region may affect gene expression in several ways that are not understood; the minimum effect will be that Z is no longer regulated by II.

To assess the relative importance of regulatory mutations and structural gene mutations in adaptive evolution, let us consider evidence from two very different kinds of investigation:

(1) Experimental studies of bacterial evolution.
(2) Studies on the relative rates of structural gene evolution, regulatory evolution and anatomical evolution in vertebrates.

BACTERIAL ADAPTATION TO NEW RESOURCES

Direct evidence for the evolutionary importance of regulatory mutations comes from experiments with bacterial populations. Bacteria are in several ways ideal organisms with which to study the mechanism of evolution. First, bacterial populations adapt rapidly in the laboratory to new situations. This is because one can work with large populations having short generation times. Second, bacteria are relatively simple genetically, having only a few thousand genes. Third, the biochemistry and genetics of some bacteria are so well known that one can hope to gain a precise molecular under-

standing of how they adapt to a well-defined change in environment. Much progress toward such an understanding has come from studies in which the environmental change is the appearance of a novel chemical.

Table 1. Examples of bacterial adaptation to new resources by means of regulatory mutations.*

Novel carbon compound	Enzyme permitting its catabolism	Genus
D-arabinose	Fucose isomerase	*Escherichia*
Arbutin	Phosphoglucosidase B	*Escherichia*
β-glycerophosphate	Alkaline phosphatase	*Escherichia*
Lactobionic acid	β-galactosidase (Z)	*Escherichia*
Lactose	β-galacotosidase (EBG)	*Escherichia*
Neolactose	β-galactosidase (Z)	*Escherichia*
Raffinose	α-galactosidase	*Escherichia*
D-arabinose	Fucose isomerase	*Klebsiella*
D-lyxose	Isomerase	*Klebsiella*
Mannitol	Arabitol dehydrogenase	*Klebsiella*
Xylitol	Ribitol dehydrogenase	*Klebsiella*
Butyramide	Acetamidase	*Pseudomonas*
Cellobiose	Phosphoglucosidase A	*Salmonella*
Rhamnose	Isomerase	*Yersinia*

*Examples taken from reviews by Hegeman and Rosenberg (1970) and Clarke (1974) and from Campbell, Lengyel and Langridge (1973).

When a bacterial population encounters a novel carbon compound rare individuals may by chance carry a mutation that permits metabolic utilisation of the compound. The mutants have a selective advantage if no other carbon source is available. Laboratory studies reveal that the primary event permitting such adaptation to a new resource is often a regulatory mutation. The regulatory mutant produces a high concentration of an enzyme that has weak activity on the new compound owing to chance chemical resemblance between the latter and the normal substrate. By virtue of having perhaps 100 times more of this enzyme than the wild-type bacteria do, the mutant can metabolise the new compound at a biologically significant rate. Table 1 lists some of the many cases in which a regulatory mutation confers the ability to utilise a new compound for growth. One of these cases is further illustrated in Figure 3.

A second stage of genetic adaptation to a novel carbon source can sometimes result from mutations affecting the amino acid sequence of a derepressed enzyme. The mutations, occuring in the gene coding for the enzyme, may alter the active site so that its reactivity with the novel analogue

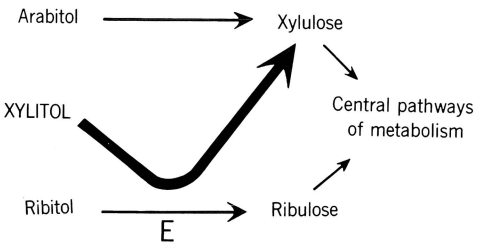


Figure 3. Metabolic pathway by which the novel compound, xylitol, is utilized by *Klebsiella* mutants. The enzyme (E), which normally functions in the ribitol pathway, slowly catalyses the conversion of xylitol to xylulose. As the enzymes for degrading xylulose already exist in wild-type *Klebsiella*, mutations that elevate the concentration of E permit *Klebsiella* to grow on xylitol. These mutations are probably not in the structural gene for E (LERNER, WU and LIN 1964, RIGBY *et al.* 1974).

increases. The altered enzyme can therefore catalyse more rapidly the metabolism of the new compound. Such mutations have been reported for three of the 15 cases of bacterial adaptation listed in Table 1.

In summary, regulatory mutations are of primary importance in permitting bacteria to utilise new resources.

RATES OF EVOLUTION

To obtain further insight into the genetic basis of evolutionary change at the organismal level, it is helpful to measure the evolutionary rates of various processes. Any type of genetic change whose evolutionary rate correlates with rate of organismal evolution could be at the basis of organismal change.

Many biologists have observed a correlation between genic similarity, estimated by protein comparisons, and organismal similarity, measured in terms of taxonomic distance. SELANDER and JOHNSON (1973) and AVISE (1974) summarised electrophoretic evidence for such a correlation. However, this correlation could result simply from the fact that both structural genes and anatomy usually evolve at fairly steady rates. If genic change and organismal change are each correlated with time, they will seem to be correlated with each other. To find out whether organismal change is dependent on structural gene mutations, one needs to compare the rates of structural gene change in taxonomic groups which have experienced contrasting rates of organismal change.

The vertebrates are well suited for such a study. Some vertebrate lineages have experienced faster rates of phenotypic evolution than others. Placental mammals, for instance, have experienced rapid organismal evolution compared to lower vertebrates, of which frogs are a typical example. Although there are thousands of frog species living today (GORHAM 1974), they are so uniform phenotypically that zoologists put them all in a single order (Anura) whereas placental mammals are divided into at least 16 orders (Table 2). The anatomical diversity represented by bats, whales, cats and people is unparallelled among frogs. Yet frogs are a much older group than placental mammals. By way of illustration, the frog genus *Xenopus* was in existence before the radiation of the placental mammals began (ESTES 1975). Thus organismal evolution has been slow in frogs relative to mammals. Hence, any type of genetic change that has occurred rapidly in mammals but slowly in frogs might be at the basis of organismal evolution. It is therefore of interest to review evidence concerning the relative rates of structural gene evolution and regulatory evolution in frogs and mammals.

Table 2. Rates of evolution in frogs and placental mammals.

Property	Frogs	Placental Mammals
Number of living species	3050*	4600
Number of orders	1	16-20
Age of the group (millions of years)	150	75
Rate of organismal evolution	Slow	Fast
Rate of albumin evolution	Standard	Standard
Rate of loss of hybridisation potential	Slow	Fast
Rate of change in chromosome number	Slow	Fast
Rate of change in number of chromosome arms	Slow	Fast

*This figure for the number of frog species known in 1970 is taken from GORHAM (1974) and may underestimate rather grossly the actual number of living species because so many species of frog are being discovered every year.

PROTEIN EVOLUTION IN FROGS AND MAMMALS

To measure rates of protein evolution, one compares the amino acid sequences of homologous proteins from species of known divergence time. Quantitative immunological comparisons provide a simple and economical way of estimating the approximate extent of sequence difference among proteins. As illustrated in Figure 4, a moderately strong correlation ($r = 0.9$) exists between degree of sequence difference and

degree of antigenic difference measured by the micro-complement fixation technique (CHAMPION *et al.* 1974, 1975). This technique was used by Maxson, Sarich and Wallace in my laboratory to compare the albumins of hundreds of frog and mammal species. Albumin is an ideal protein for evolutionary studies because it evolves quite rapidly and consists of one polypeptide chain that is 580 amino acids long (BROWN 1975); thus the albumin gene is equivalent in size to the combined genes for cytochrome c, myoglobin, the hemoglobin chains (alpha and beta) and the fibrinopeptides A and B.

Albumin seems to have evolved no faster in mammals than in frogs (WALLACE, MAXSON and WILSON 1971, WALLACE and WILSON 1972, WALLACE, KING and WILSON 1973, MAXSON and WILSON 1975, MAXSON, SARICH and WILSON 1975). As a consequence of this remarkable situation, species which are similar enough in anatomy and way of life to be included within a single genus of frogs (e.g. *Rana*) can differ as much in their albumins as does a bat from a whale.

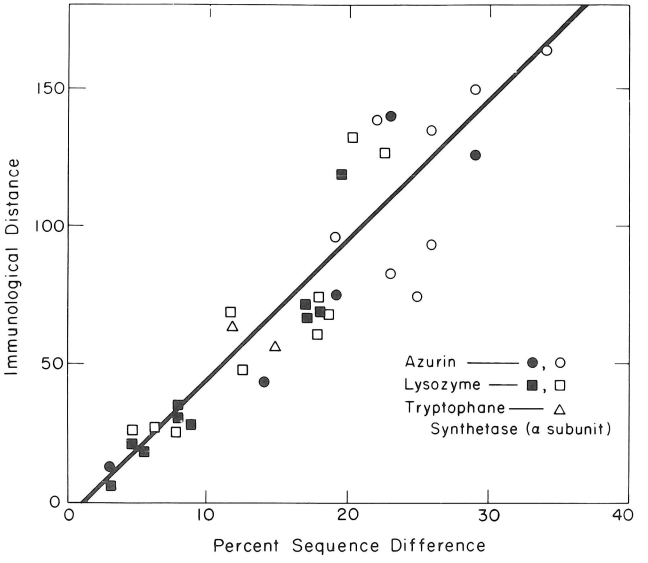


Figure 4. Dependence of immunological distance on percent sequence difference among bacterial azurins, bird lysozymes, and α subunits of bacterial tryptophan synthetases (from CHAMPION *et al.* 1975).

Other proteins have been studied less extensively although the results are consistent with those obtained from albumin. The beta chains of hemoglobin from two species of *Rana* differ in their amino acid sequences by at least 29 substitutions,

which is a greater difference than that usually found between any two orders of placental mammals (BALDWIN and RIGGS 1974). Additional hemoglobin evidence (MAXSON and WILSON 1975) as well as electrophoretic evidence obtained with numerous enzymes (CASE, HANELINE and SMITH 1975) agrees with the hypothesis that anatomically similar frogs can differ greatly at the protein sequence level.

DNA EVOLUTION IN FROGS AND MAMMALS

DNA annealing studies with non-repeated sequences have been conducted with a variety of mammals (KOHNE *et al.* 1972, HOYER *et al.* 1972, BENEVENISTE and TODARO 1974) and two sibling species of frog belonging to the archaic genus *Xenopus* (GALAU and DAVIDSON 1976). The latter two species are so similar at the organismal level that they were both included in *Xenopus laevis* (as the subspecies *X. l. laevis* and *X. l. borealis*) until very recently (FISCHBERG, pers. commun.). The *Xenopus* difference (ΔT_m = 12°) is larger than that found between the DNA of humans and New World monkeys. These limited studies give no reason to think that sequence evolution at the DNA level has been slower in frogs than in mammals.

REGULATORY EVOLUTION IN FROGS AND MAMMALS

Two lines of evidence are suggestive of sluggish regulatory evolution in frogs as compared to mammals.

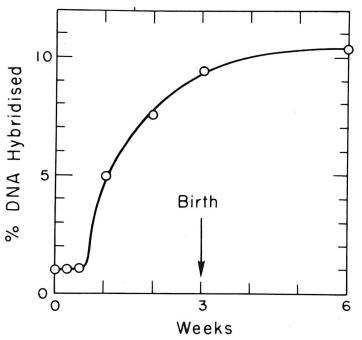


Figure 5. Increase in diversity of RNA transcripts of DNA during mouse development (from CHURCH and BROWN 1972).

To appreciate the first line of evidence, one needs to consider interspecific hybridisation. Although there are many natural barriers to fertilisation of an egg by sperm of another species, these are usually not absolute. Supposing an interspecific zygote is formed, one can ask what chance it has of developing into a healthy adult. Embryonic development involves an orderly program of expression of many genes that were inactive in the zygote (Figure 5). If the two genomes in an inter-

specific zygote are similarly programmed, so that a given block of genes will be turned on at the same time in one genome as in the other, orderly development of a hybrid organism can be expected. However, should the patterns of gene activation differ, the probability of an interspecific zygote developing successfully would be low. In accordance with this view, organismal hybrids derived from extremely different parental species often show signs of breakdowns in gene regulation (OHNO 1969, WHITT CHILDERS and CHO 1973).

If regulatory evolution has proceeded slowly in frogs relative to mammals, one would expect that frog species should retain the ability to hybridise with one another much longer than mammals do. Since the rate of albumin evolution in frogs has been equal to that in mammals, one expects to find small albumin distances among mammals capable of hybridising whereas among hybridisable frogs one should encounter large immunological distances. The albumin results are in accordance with this expectation (Figure 6).

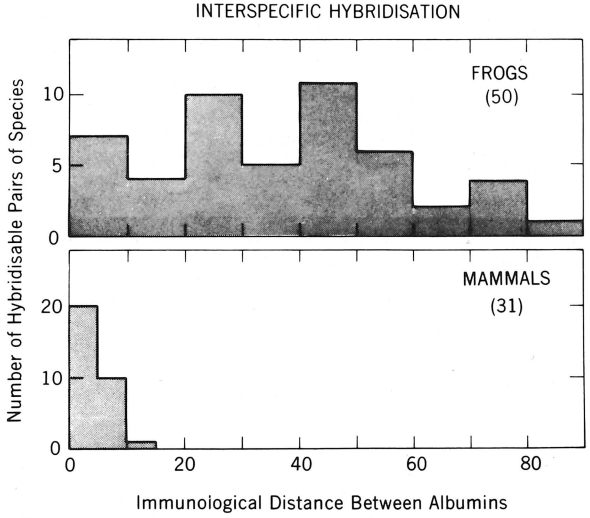


Figure 6. Immunological distances between albumins of species pairs capable of producing viable hybrids. Thirty-one such pairs of placental mammal species and 50 pairs of frog species were investigated (from WILSON, MAXSON and SARICH 1974).

Chromosomal studies provide a second line of evidence, consistent with slower regulatory evolution in frogs than in mammals. Placental mammals have experienced far more rapid karyotypic change than have frogs. This is evident from albumin studies on hundreds of species of known karyotype (WILSON, SARICH and MAXSON 1974). The albumin immunological distance at

which there is a 50% chance that two species will differ in chromosome number is about 6 units for mammals and about 120 units for frogs (Figure 7). Changes in chromosome number are

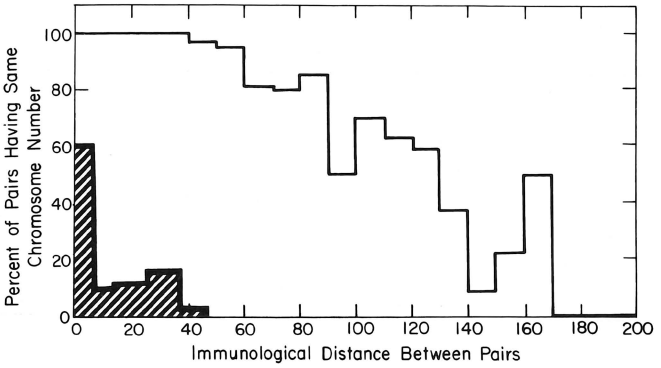


Figure 7. Proportion of species pairs having identical chromosome number as a function of the immunological distance between the albumins of the pairs. The light stippled histogram summarises the results for 373 different pairs of frog species. The hatched histogram summarises the results for 318 different pairs of placental mammal species (from WILSON, SARICH and MAXSON 1974).

brought about by fusion or fission events, as illustrated in Figure 8a. A comparable analysis was done with the number of chromosomal arms. Changes in arm number result mainly from inversions as shown in Figure 8b. The rates at which arm number has changed turn out to be very similar to the rates of change in chromosome number (WILSON, SARICH and MAXSON, 1974). It is inferred that these two distinct types of gene rearrangement have each been evolving an order of magnitude faster in placental mammals than in frogs.

The studies on rates of evolution in frogs and mammals suggest that sequence changes in structural genes occur independently of organismal change, whereas regulatory changes, as manifested by studies of karyotype and hybrid viability, evolve in parallel with organismal change. To assess the significance of these findings, it is desirable to examine other groups with contrasting rates of evolution.

EVOLUTION IN HUMANS AND CHIMPANZEES.

The comparison of human and chimpanzee macromolecules is of interest in this connection. Because of major differences at the organismal level, these two species are classified in different taxonomic families. Yet, at the macromolecular sequence level, humans and chimpanzees are extraordinarily simi-

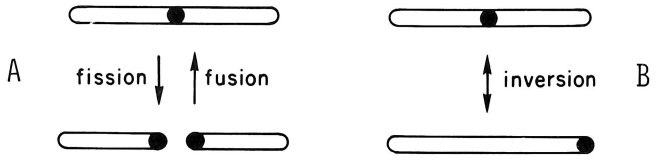


Figure 8. Mechanisms by which evolutionary changes occur in (a) chromosome number and (b) number of chromosome arms. The addition of heterochromatin can also cause changes in arm number.

lar. Species within a genus of mice, frogs or flies can differ more from each other than does a human from a chimpanzee, as is evident from the DNA studies summarised in Table 3. A similar

Table 3. DNA comparisons made by measuring the thermal stability of hybrid double strands.

Species compared	Percent sequence difference in DNA*	Level of taxonomic difference	Reference
HOMINOIDS			
Human with chimpanzee	1.1	Family	Kohne *et al.* (1972); Hoyer *et al.* (1972); King & Wilson (1975).
MICE			
Mus musculus with *M. cervicolor*	5	Species	Rice (1972).
FROGS			
Xenopus laevis with *X. borealis*	12	Species	Galau & Davidson (1976).
FLIES			
Simulium pictipes with *S. venustum*	11	Species	Sohn, Rothfels & Straus (1975).
Drosophila melanogaster with *D. salmon*	19	Species	Laird (1973).

*Estimated from studies with non-repetitive DNA and the assumption that a 1% difference in base sequence lowers thermal stability by 1°C.

result is obtained from extensive protein comparisons, which are illustrated in Figure 9. It seems probable that, at the struc-

tural gene level, chimpanzees and humans are as similar as a pair of sibling species (KING and WILSON 1975).

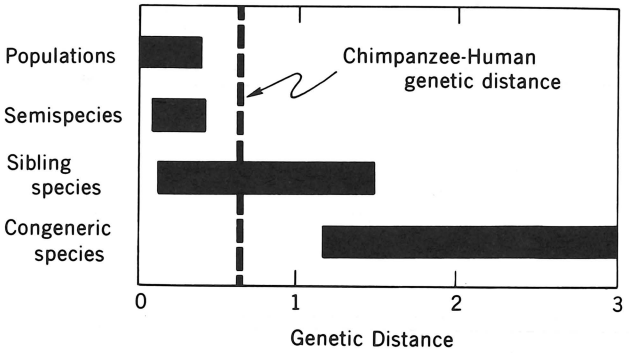


Figure 9. The genetic distance between humans and chimpanzees compared to that between other organisms. These estimates of genetic distance are based on electrophoretic comparison of many proteins. One unit of genetic distance means that there is an average of one electrophoretically detectable substitution per polypeptide compared. The bars indicate the range of genetic distances found among various populations, semispecies, sibling species and congeneric species of a wide variety of organisms including fruit flies, horseshoe crabs, salamanders, lizards, fishes, bats and rodents. Semispecies are not quite full species, because limited gene flow occurs between them. Sibling species are so alike in morphology that experts thought they were the same species until chromosomal, biochemical or interbreeding tests were conducted. Congeneric species are those that belong to the same genus; for the purpose of the figure, we excluded sibling species from the congeneric category (from KING and WILSON 1975).

An evolutionary perspective further illustrates the contrast between the results of the molecular and organismal approaches. Since the common ancester of the two species lived, the chimpanzee lineage has evolved slowly relative to the human lineage, in terms of anatomy and adaptive strategy. This concept is illustrated in Figure 10. However, phylogenetic analysis of the sequence comparisons among chimpanzees, humans and other primates indicates that the two lineages have undergone approximately equal amounts of sequence change in their macromolecules. Hence, the major adaptive change which took place in the human lineage was probably not accompanied by accelerated DNA or protein sequence evolution.

What then is the genetic basis for the evolution of humans from apes? The organismal differences between apes and humans might result chiefly from genetic changes in a few

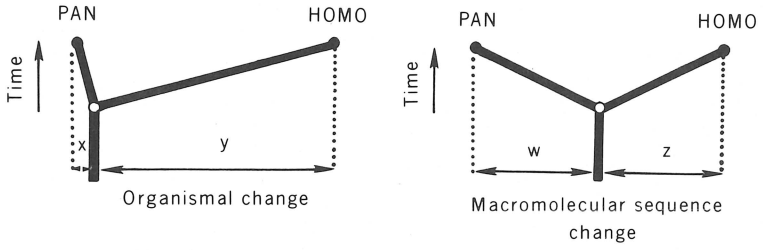


Figure 10. The contrast between biological evolution and molecular evolution since the divergence of the human and chimpanzee lineages. As the left diagram shows, more biological change has taken place in the human lineage (y) than in the chimpanzee lineage (x). Protein and nucleic acid evidence indicate that as much change has occurred in chimpanzee genes (w) as in human genes (z) -- right diagram (from KING and WILSON 1975).

regulatory systems. The idea that regulatory mutations have had a major role in human evolution is consistent with the old observation that adult humans resemble foetal apes in some respects. The retention of foetal patterns of gene expression during childhood may have played a part in evolving such human features as our large brain, our small jaws and canine teeth, our naked skin and our upright posture (GOULD 1975).

Gene rearrangement could be an important source for such changes in gene regulation during human evolution. Although humans and chimpanzees do not differ greatly in chromosome number, having 46 and 48 chromosomes, respectively, the arrangement of genes differs in the two species. Only a small proportion of the chromosomes have identical banding patterns in these two hominoids. The banding studies indicate that at least ten large inversions and translocations and one chromosomal fusion have occurred since the two lineages separated. Further evidence, summarised by KING and WILSON (1975), that humans and chimpanzees differ considerably in chromosomal organisation emerges from studies in which a purified nucleic acid fraction was annealed to chromosomes *in situ*. The chromosomal sites at which annealing occurred were distributed quite differently in the two hominoids, indicating different gene arrangements on the chromosomes.

It is therefore proposed, as a working hypothesis, that chimpanzee and human genes are remarkably similar simply because the species separated rather recently (SARICH and WILSON 1973, GOODMAN 1974), whereas the large organismal differences are due to rapid regulatory evolution in the human lineage.

EVOLUTION IN OTHER ORGANISMS

Many other examples of contrasts between genic similarity and organismal similarity are known. Space does not permit detailed review of these findings. Suffice it to say that such contrasts have been encountered within a wide variety of taxonomic groups, ranging from bacteria (STANIER *et al.* 1970) and ciliates (BORDEN, WHITT and NANNEY 1973) to snails (GOULD, WOODRUFF and MARTIN 1974), fishes (TURNER 1974), frogs (MAXSON and WILSON 1974, 1975), reptiles (MAO and DESSAUER 1971) and birds (NOLAN *et al.* 1975). It may therefore be a general rule that organismal evolution and structural gene evolution go on at virtually independent rates. In line with this suggestion is the intriguing evidence that speciation can occur without alteration of structural genes in both insects (BUSH 1975) and flowering plants (GOTTLIEB 1976).

To date, there is little published evidence for a correlation between organismal evolution and evolution at the level of chromosome organisation or hybrid viability for groups other than frogs and placental mammals. Some evidence is available for birds. Relative to placental mammals, this group has experienced slow anatomical evolution in the past 30 million years. During this period, birds have also experienced slow loss of hybridisation potential and slow chromosomal evolution (PRAGER and WILSON 1975). Additional studies of this sort are in progress with other vertebrate groups. Similar tests can be done with several invertebrate and plant groups having good fossil records. Although such testing has scarcely begun. It is apparent already that anatomically conservative groups are often conservative with respect to both karyotype and hybridisation potential (WILSON, CASE and KING, unpublished work).

CONCLUSIONS AND PROSPECTS

The following conclusions are drawn tentatively from the findings reviewed above on the mechanism of bacterial adaptation and on rates of evolution. Evolution at the organismal level may depend primarily on regulatory mutations, which alter patterns of gene expression. Mutations affecting the arrangement of genes on chromosomes may be a common source of these altered patterns of gene expression. By contrast, sequence changes in structural genes may be of secondary importance; this process is not tightly geared to organismal evolution and tends to go on relentlessly at about the same rate in all organisms.

To test these tentative conclusions, it is important to make deeper studies of the genetic and biochemical mechanisms underlying bacterial adaptation. More thorough studies of rates of evolutionary change in anatomy, genes, chromosomes and hybrid viability would also be valuable. In order to examine quantitatively the relationship between molecular evolution and organismal evolution, it is important to use numerical methods for estimating degree of organismal resemblance. Until we know how much more rapidly placental mammals have evolved

than frogs have, at the organismal level, one cannot know how strong the correlation is between organismal change and change in chromosomes or hybridisation potential. It is also important for evolutionary geneticists to become familiar with current research on the organisation of eucaryotic genomes and on the molecular biology of gene expression, especially during embryogenesis. One may anticipate that interaction with these areas of research will stimulate the development of better methods for estimating degree of difference between patterns of gene expression.

It does not follow that comparative studies of amino acid and nucleotide sequences should be abandoned. Evolutionary biologists can take advantage of the finding that sequences evolve at fairly steady rates that are virtually independent of rates of organismal evolution (SARICH and WILSON 1973, MAXSON, SARICH and WILSON 1975, FITCH 1976). Proteins and nucleic acids may become valuable tools for elucidating objectively the order of branching of lineages and for estimating the approximate times of divergence of lineages (SARICH 1973, MAXSON and WILSON 1975). Information of this sort should provide a framework that enables evolution at other levels to be studied more effectively, especially in groups whose fossil record is poor or lacking.

ACKNOWLEDGEMENTS

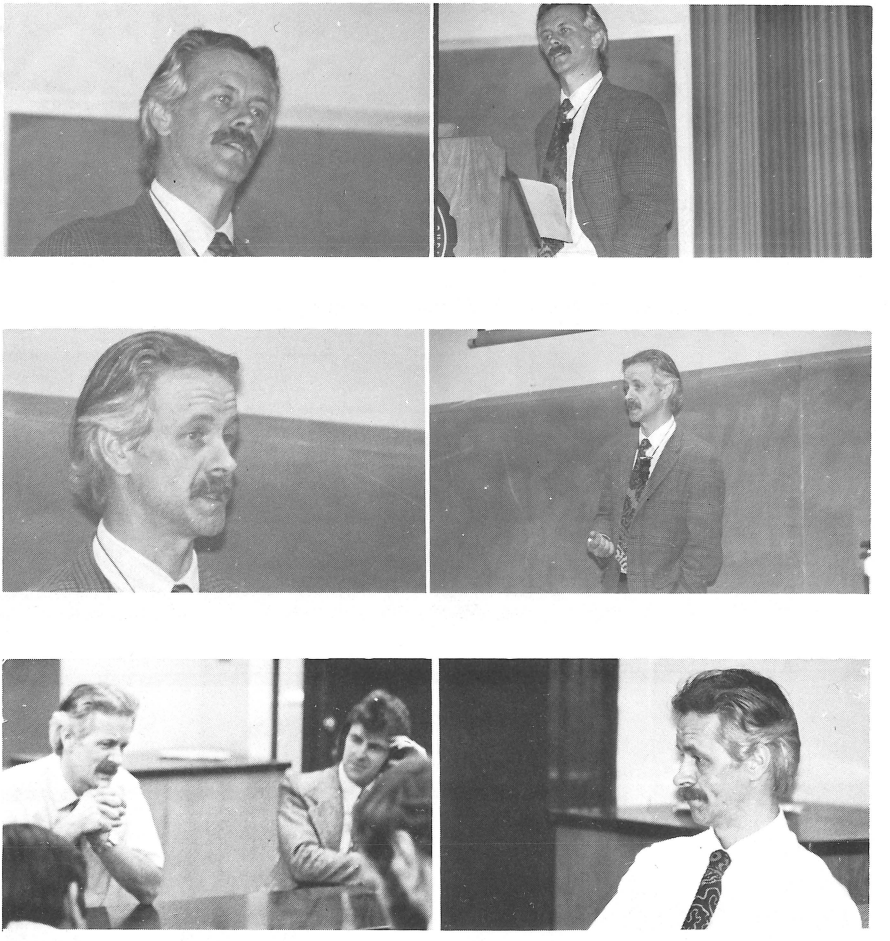
I thank B. N. Ames, S. M. Beverley, S. S. Carlson, V. M. Sarich, and T. J. White for helpful discussion.

LITERATURE CITED

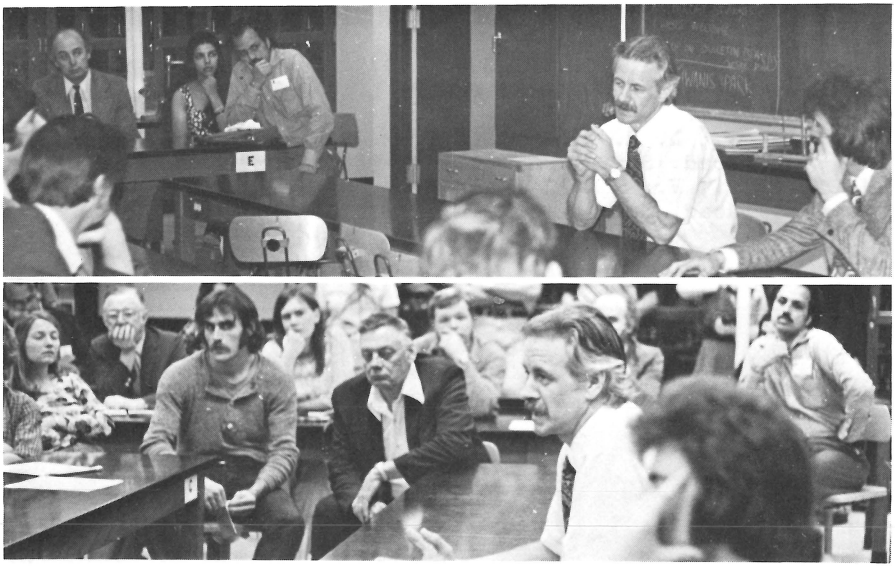
AVISE, J. C. 1974. Systematic Zool. 23:465-481.
BAHN, E. 1971. Hereditas 67:79-82.
BALDWIN, T. O. and A. RIGGS. 1974. J. Biol. Chem. 249:6110-6118.
BENEVENISTE, R. E. and G. J. TODARO. 1974. Proc. Nat. Acad. Sci. U.S. 71:4513-4518.
BERTRAND, K., L. KORN, F. LEE, T. PLATT, C. L. SQUIRES, C. SQUIRES and C. YANOFSKY. 1975. Science 189:22-26.
BORDEN, D.,G. S. WHITT and D. L. NANNEY. 1973. J. Protozool. 20:693-700.
BUSH, G. L. 1975. Ann. Rev. Ecol. Systematics 6:339-364.
CAMPBELL, J. H., J. A. LENGYEL and J. LANGRIDGE. 1973. Proc. Nat. Acad. Sci. U.S. 70:1841-1845.
CASE, S. M., P. G. HANELINE and M. F. SMITH. 1975. Systematic Zoology 24:(in press).
CHAMPION, A. G., E. M. PRAGER, D. WACHTER and A. C. WILSON. 1974. In Biochemical and Immunological Taxonomy of Animals (Wright, C. A., ed.) Academic Press, London. pp. 397-416.
CHAMPION, A. B., K. L. SODERBERG, A. C. WILSON and R. P. AMBLER. 1975. J. Molec. Evolution:(in press).
CHURCH, R. B. and I. R. BROWN. 1972. Results Probl. Cell Differentiation 3:11-24.

CLARKE, P. H. 1974. In Evolution the Microbial World (Carlile, M. J. and J. J. Skehel, Eds.) Cambridge Univ. Press. pp. 183-187.
ESTES, R. 1975. Nature 254:48-50.
FITCH, W. M. 1976. In Molecular Basis of Biological Evolution (Ayala, F. J. Ed.) Sinauer Associates, Sunderland, Mass. (in press).
GALAU, G. and E. H. DAVIDSON. 1976. In Molecular Basis of Biological Evolution (Ayala, F. J. Ed.) Sinauer Associates, Sunderland, Mass. (in press).
GOODMAN, M. 1974. Ann. Rev. Anthropol. 3:203-228.
GORHAM, S. W. 1974. Checklist of World Amphibians up to January 1, 1970. New Brunswick Museum, St. John, Canada.
GOTTLIEB, L. 1976. In Molecular Basis of Biological Evolution (Ayala, F. J., Ed.) Sinauer Associates, Sunderland, Mass., (in press).
GOULD, S. J. 1975. Natural History 84:18-22.
GOULD, S. J., D. S. WOODRUFF and J. P. MARTIN. 1974. Systematic Zool. 23:518-535.
HEGEMAN, G. D. and S. L. ROSENBERG. 1970. Ann. Rev. Microbiol. 24:429-462.
HOYER, B. H., N. W. VAN DE VELDE, M. GOODMAN and R. B. ROBERTS. 1972. J. Human Evolution 1:645-649.
KING, M. C. and A. C. WILSON. 1975. Science 188:107-116.
KOHNE, D. E., J. A. CHISCON and B. H. HOYER. 1972. J. Human Evolution 1:627-644.
LAIRD, C. D. 1973. Ann. Rev. Genet. 3:177-204.
LERNER, S. A., T. T. WU and E. C. C. LIN. 1964. Science 146: 1313-1315.
MAO, S.-H. and H. C. DESSAUER. 1971. Comp. Biochem. Physiol. 40A:669-680.
MAXSON, L. R., V. M. SARICH and A. C. WILSON. 1975. Nature 255: 397-400.
MAXSON, L. R. and A. C. WILSON. 1974. Science 185:66-68.
MAXSON, L. R. and A. C. WILSON. 1975. Systematic Zool. 24:1-15.
NOLAN, R. A., A. H. BRUSH, N. ARNHEIM and A. C. WILSON. 1975. Condor 77:154-159.
OHNO, S. 1969. In Heterospecific Genome Interaction (Defendi, V., Ed.) Wistar Instit. Press, Philadelphia. pp. 137-150.
PRAGER, E. M. and A. C. WILSON. 1975. Proc. Nat. Acad. Sci. U. S. 71:200-204.
RICE, N. R. 1972. Brookhaven Symp. Biol. 23:44-76.
RIGBY, P. W. J., B. D. BURLEIGH and B. S. HARTLEY. 1974. Nature 251:200-204.
SARICH, V. M. 1973. Nature 245:218-220.
SARICH, V. M. and A. C. WILSON. 1973. Science 71:1144-1147.
SELANDER, R. K. and W. E. JOHNSON. 1973. Ann. Rev. Ecol. Systematics 4:75.
SOHN, U.-I., K. H. ROTHFELS and N. A. STRAUSS. 1975. J. Molec. Evol. 5:75-85.
STANIER, R. Y., D. WACHTER, C. GASSER and A. C. WILSON. 1970. J. Bacteriol. 102:351-362.
TURNER, B. J. 1974. Evolution 28:281-294.
WALLACE, B. and T. L. KASS. 1974. Genetics 77:541-558.
WALLACE, D. G., L. R. MAXSON and A. C. WILSON 1971. Proc. Nat.

Acad. Sci. U. S. 68:3127-3129.
WALLACE, D. G., M.-C. KING and A. C. WILSON. 1973. Systematic Zool. 22:1-13.
WALLACE, D. G. and A. C. WILSON. 1972. J. Molec. Evol. 2:72-86.
WHITT, G. S., W. F. CHILDERS and P. L. CHO. 1973. J. Hered. 64:55-61.
WILSON, A. C., L. R. MAXSON and V. M. SARICH. 1974. Proc. Nat. Acad. Sci. U. S. 71:2843-2847.
WILSON, A. C., V. M. SARICH and L. R. MAXSON. 1974. Proc. Nat. Acad. Sci. U. S. 71:3028-3030.



Allan C. Wilson at the Symposium (with R. Flavell, bottom left)



Allan Wilson with discussion groups

Allan Wilson answering questions after his lecture

Paul Bolen G. Kikudome, D. Miles Don Duvick