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The Importance of Gene Rearrangement in Evolution: Evidence from Studies on Rates of Chromosomal, Protein, and Anatomical Evolution

(mammals/frogs/albumin/microcomplement fixation/chromosome number/mechanism of evolution)

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ABSTRACT **We have compared the relative rates of protein evolution and chromosomal evolution in frogs and mammals. The average rate of change in chromosome number has been about 20 times faster in mammals than in frogs. Whereas it takes only 3.5 million years, on the average, for a pair of mammal species to develop a difference in chromosome number, the corresponding period for frogs is 70 million years. In contrast, the rate of protein evolution in mammals has been roughly equal to that in frogs. The rapid rate of gene rearrangement in mammals parallels both their rapid anatomical evolution and their rapid evolutionary loss of the potential for interspecific hybridization. Thus, gene rearrangements may be more important than point mutations as sources for evolutionary changes in anatomy and way of life.**

There must be a molecular basis for the differences in anatomy and way of life among organisms. Nonetheless, despite the vast effort devoted in recent years to the study of nucleic acid and protein evolution, serious problems arise when one tries to reconcile organismal evolution with macromolecular evolution (1–3). We now present evidence that there may be a close parallel between chromosomal evolution and organismal evolution. Attention is therefore focused on the idea that the phenomenon of gene rearrangement may be at the basis of organismal evolution.

This idea emerges from consideration of the processes of organismal, chromosomal, and molecular evolution in frogs as compared to mammals. As recently pointed out, rates of protein evolution in frogs have been very similar to those in mammals even though organismal evolution has proceeded much more slowly in frogs than in mammals (1–3). The contrast was explained by postulating rapid evolution of mammalian regulatory systems (3), guided by natural selection, while the anuran adaptive zone remained a far more conservative one. This necessarily implies that protein evolution in both groups occurs independently of whether or not other evolutionary changes are taking place. One may then ask if these postulated changes in regulatory systems, occurring so much more rapidly in mammals, are reflected at any level other than that of the organism itself. We suggest that this level is that of chromosome structure. The supportive evidence comes from an estimation of the relative rates of chromosome evolution and protein evolution in frogs and mammals.

MATERIALS AND METHODS

Antisera were made to the purified albumins of 28 frog and 36 mammal species of known chromosome number. Each antiserum was tested for reactivity with serum or plasma from numerous species of known chromosome number by means of the quantitative microcomplement fixation method. Although space does not permit the listing of all 236 of the species examined, an indication of their taxonomic variety is given in Table 1. The details of albumin purification, antiserum production, and immunological distance measurement have been given (2–6). Immunological distances are approximately equal to the number of amino-acid sequence differences between two albumins (7).

Although we studied primarily albumin, protein evolution proceeds with sufficient regularity (8) to make us confident that species whose albumins differ greatly will also differ substantially at other loci as well. Electrophoretic and DNA hybridization measurements of genetic distance (9–11) correlate well ($r = 0.8$ and $r = 0.9$, respectively) with immunological distances among the albumins of the same species (3, 7). Hence, we are confident that albumin immunological distances are indicative of the overall degree of sequence resemblance among the genomes of the species compared.

RESULTS

Mammals. We compared the albumins of 318 species pairs representing 8 orders of placental mammals (Table 1). For each species pair studied we noted whether the two species had identical or different chromosome numbers and determined the immunological distance between their albumins. Species whose albumins differ by more than 6 units usually have different chromosome numbers. This is evident from the solid black histogram (Fig. 1), which summarizes our results with mammals. Mammalian species whose albumins differ by 6 units have a 50% chance of differing in chromosome number.

Frogs. A radically different result was obtained by studying the albumins of 373 frog species pairs in the same way. As indicated in Fig. 1, frogs whose albumins differ by 6 units always have the same chromosome number. Indeed, the albumin immunological distance at which there is a 50% chance that two frogs will differ in chromosome number is roughly 120 units.

Fundamental Number. A similar picture emerges from considering the number of chromosomal arms, i.e., the "fundamental number," rather than the number of chromosomes. The albumin immunological distance at which there is a 50% chance that two species will differ in fundamental number is roughly 4 units for mammals and 120 units for frogs. The mammalian value is not precise because there is uncertainty

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TABLE 1. *Variety of species of known chromosome number whose albumins were compared*

Taxonomic group	No. of antisera*	No. of species pairs examined†
Frogs		
Bufonidae	7	95
Hylidae	16	180
Ranidae	5	98
Mammals		
Artiodactyla	3	31
Carnivora	6	36
Cetacea	1	1
Chiroptera	6	12
Perissodactyla	1	2
Primates	15	191
Proboscidea	1	1
Rodentia	6	44

* Some of the data obtained with many of these antisera have been published (1–4, 6–8, 32–36).

† Altogether we examined 93 species of mammals and 143 species of frogs. We did not compare the albumins of all possible pairs of these species (i.e., 14,431 pairs), owing in part to the fact that antisera were available to only some of them. For mammals, we give only the number of pairs whose albumins were found to differ by no more than 40 immunological distance units. We have not attempted a compilation for species pairs differing by more than 40 units because the number of such pairs is very large and, furthermore, a plateau value is reached in the histogram (Fig. 1) at about 20 units.

about the exact number of chromosomal arms in many species of mammals.

Karyologists have pointed out that artiodactyls are unusual among mammals in having undergone few evolutionary changes in fundamental number. Our findings agree with this. The albumin immunological distance at which there is a 50% chance that two artiodactyls will differ in fundamental number is roughly 15 units.

DISCUSSION

Rates of Chromosomal Evolution. Our results indicate that evolutionary changes in chromosome number have proceeded roughly 20 times faster in mammals than in frogs. This inference is drawn from estimation of the albumin immunological distance at which there is a 50% chance that a pair of species will have identical chromosome numbers. The estimates made by inspection of Fig. 1 are 120 units for frogs and 6 units for placental mammals. One should also bear in mind the evidence that albumin evolution proceeds with considerable regularity (3, 8) and that the average rate of albumin evolution in frogs appears to be equal to that in placental mammals (1, 2, 4). This rate is 1.7 units/million years. From this, we may calculate the rate at which differences in chromosome number between species evolve. For frogs we estimate that it generally takes 120/1.7, i.e., 70 million years for a difference in chromosome number to develop between two species, whereas for mammals the average time required is 6/1.7, i.e., 3.5 million years. Similar calculations show that the average rate of evolutionary change in fundamental number has been over 20 times greater for placental mammals than for frogs.

Evolutionary changes in chromosome number could result

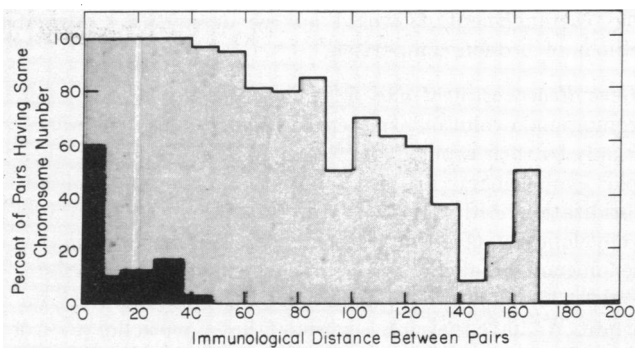

FIG. 1. Fraction of species pairs having identical chromosome number as a function of the immunological distance between the albumins of the pairs. The *light stippled histogram* summarizes the results for 373 different pairs of frog species. The *solid black histogram* summarizes the results for 318 different pairs of mammal species. The chromosome number data were taken from refs. 37–41.

from either the rearrangement, or the loss or gain, of genetic material. As most mammals, regardless of their chromosome number, have 6 pg of DNA per cell and most of the frogs examined have 10 pg of DNA per cell (12, 13), gene rearrangement events are probably responsible for most evolutionary changes in chromosome number (14). Most evolutionary changes in fundamental number are probably brought about in this way also. It therefore appears that evolutionary changes in gene arrangement have occurred far faster in mammals than in frogs.

Relationship Between Chromosomal Evolution and Anatomical Evolution. The rapid chromosomal evolution in mammals parallels their rapid anatomical evolution. However, as noted above, there is no indication that protein evolution has been accelerated in mammals.

A contrast between protein evolution and chromosomal evolution is also evident from studies conducted by population geneticists. Although it is rare to find cases of intraspecific variation in chromosome number, it is now well established that populations often exhibit other types of karyotypic variation, for example inversion or translocation of small chromosomal segments (15). Inversion polymorphisms have been especially well documented in many species of fruit flies (*Drosophila*). Intraspecific karyotypic variation also occurs in many other insects (16) as well as in many vertebrates (17). Protein studies have recently been conducted with some of these species. It is remarkable that the geographic pattern of allelic variation at loci coding for proteins usually contrasts with the pattern of chromosomal variation (18, 19). Island populations, for example, may be chromosomally distinct from mainland populations despite being virtually identical in regard to protein allelic frequencies.

Evolutionary biologists used to think that a genetic revolution accompanies the process of speciation (20, 21). However, there are now numerous reports that closely related species, though karyotypically distinct, can be extremely alike at the protein level (22–25). Such reports make some workers tend to doubt that a genetic revolution accompanies speciation. The evidence given above for frogs and mammals, however, implies that studies at the protein level may not be relevant to the question of whether such a genetic revolution occurs during speciation. If the postulated revolution occurs at the level of

gene rearrangement, it would not be detected by current methods of protein comparison.

Gene Rearrangement and Regulatory Evolution. The rapid chromosomal evolution experienced by mammals is paralleled not only by their rapid anatomical evolution but also by their rapid evolutionary loss of the potential for interspecific hybridization. Although the nature of the molecular barriers to development of an interspecific zygote is not known, the phenomenon of allelic repression, which occurs in extreme hybrids (26), shows that regulatory barriers may be very important. We have therefore suggested that evolutionary loss of the ability of two species to hybridize probably results from the accumulation of incompatibilities between the two systems for regulating the expression of genes during embryonic development (3). Thus, mammals appear to have undergone both rapid regulatory evolution and rapid rearrangement of genes. This correlation may indicate that gene rearrangement provides an important means of achieving new patterns of regulation. Although little is known about the mechanisms for regulating gene expression in vertebrates, molecular biologists are now giving much attention to the organization of genes on chromosomes (27–29).

The idea that gene rearrangements may contribute significantly to adaptive evolution is not new, having been discussed at length by Ford (30), in his development of the "supergene" concept, and recently by Soulé (31). It may be useful, then, to regard adaptive evolution as resulting primarily from changes in the expression of genes relative to one another rather than from amino-acid substitutions in the products of those genes. Adaptation is probably a complex process requiring new interactions among many genes. The reshuffling of genes may be an important mechanism by which new interactions can occur.

The hypothesis that gene rearrangement is a key factor in organismal evolution can be tested. The hypothesis predicts that the number of gene rearrangements should be correlated with the degree of morphological evolution exhibited by a lineage over time. Thus, morphologically conservative forms should show relatively less change from an ancestral karyotype as compared to forms that have undergone rapid adaptive change. There is, therefore, a need for high-resolution chromosome studies on species representing lineages with known rates of anatomical evolution.

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1. Wallace, D. G., Maxson, L. R. & Wilson, A. C. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 3127–3129.
2. Wallace, D. G., King, M.-C. & Wilson, A. C. (1973) *Syst. Zool.* **22**, 1–13.
3. Wilson, A. C., Maxson, L. R. & Sarich, V. M. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 2843–2847.
4. Maxson, L. R. (1973) Ph.D. Dissertation in Genetics, University of California, Berkeley, and California State University, San Diego, 1–253.
5. Wallace, D. G. & Wilson, A. C. (1972) *J. Mol. Evol.* **2**, 72–86.
6. Sarich, V. M. (1969) *Syst. Zool.* **18**, 286–295 and 416–422.
7. Maxson, L. R. & Wilson, A. C. (1974) *Science* **185**, 66–68.
8. Sarich, V. M. & Wilson, A. C. (1973) *Science* **179**, 1144–1147.
9. Selander, R. K. & Johnson, W. E. (1973) *Annu. Rev. Ecol. Systematics* **4**, 75–91.
10. Kohne, D. E., Chiscon, J. A. & Hoyer, B. H. (1972) *J. Human Evol.* **1**, 627–644.
11. Hoyer, B. H., van de Velde, N. W., Goodman, M. & Roberts, R. B. (1972) *J. Human Evol.* **1**, 645–650.
12. Sparrow, A. H., Price, H. J. & Underbrink, A. G. (1972) *Brookhaven Symp. Biol.* **23**, 451–494.
13. Bachman, K., Goin, O. B. & Goin, C. S. (1972) *Brookhaven Symp. Biol.* **23**, 419–450.
14. Ohno, S. (1970) *Evolution by Gene Duplication* (Springer-Verlag, New York), 1–160.
15. White, M. J. D. (1969) *Annu. Rev. Genet.* **3**, 75–98.
16. John, B. & Lewis, K. R. (1966) *Science* **152**, 711–721.
17. Patton, J. L. (1972) *Evolution* **26**, 574–586.
18. Ayala, F. J., Powell, J. R. & Tracey, M. L. (1972) *Genet. Res.* **20**, 19–42.
19. Nevo, E., Kim, Y. J., Shaw, C. R. & Thaeler, C. S., Jr. (1974) *Evolution* **28**, 1–23.
20. Dobzhansky, Th. (1959) *Cold Spring Harbor Symp. Quant. Biol.* **24**, 15–27.
21. Mayr, E. (1963) *Animal Species and Evolution* (Harvard University Press, Cambridge), 1–797.
22. Nevo, E. & Shaw, C. R. (1972) *Biochem. Genet.* **7**, 235–241.
23. Patton, J. L., Selander, R. K. & Smith, M. H. (1972) *Syst. Zool.* **21**, 263–270.
24. Johnson, W. E., Selander, R. K., Smith, M. H. & Kim, Y. J. (1972) *Studies in Genetics VII. Univ. Texas Publ.* **7213**, 297–305.
25. Selander, R. K., Kaufman, D. W., Baker, R. J. & Williams, S. L. (1974) *Evolution*, in press.
26. Whitt, G. S., Childers, W. F. & Cho, P. L. (1973) *J. Hered.* **64**, 55–61.
27. Davidson, E. H. & Britten, R. J. (1973) *Quart. Rev. Biol.* **48**, 563–613.
28. Darnell, J. E., Jelinek, W. R. & Molloy, G. R. (1973) *Science* **181**, 1215–1221.
29. Thomas, C. A. (1973) in *Regulation of Transcription and Translation in Eukaryotes*, ed. Bautz, E. K. F. (Springer-Verlag, Berlin), pp. 1–30.
30. Ford, E. B. (1965) *Genetic Polymorphism* (MIT Press, Cambridge), 17–25.
31. Soulé, M. (1973) *Annu. Rev. Ecol. Syst.* **4**, 165–187.
32. Sarich, V. M. & Wilson, A. C. (1967) *Proc. Nat. Acad. Sci. USA* **58**, 142–148.
33. Sarich, V. M. & Wilson, A. C. (1967) *Science* **158**, 1200–1203.
34. Sarich, V. M. (1970) in *Old World Monkeys*, eds. Napier, J. R. & Napier, P. H. (Academic Press, New York), pp. 175–226.
35. Sarich, V. M. (1972) *Biochem. Genet.* **7**, 205–212.
36. Sarich, V. M. (1973) *Nature* **245**, 218–220.
37. Duellman, W. E. (1967) *Syst. Zool.* **16**, 38–43.
38. Hsu, T. C. & Benirschke, K. (1967–1971) *Atlas of Mammalian Chromosomes* (Springer-Verlag, New York), Vols. 1–5.
39. Benirschke, K., ed. (1969) *Comparative Mammalian Cytogenetics* (Springer-Verlag, New York), 1–473.
40. Bogart, J. P. (1972) in *Evolution in the Genus Bufo*, ed. Blair, W. F. (U. Texas Press, Austin), pp. 171–195.
41. Bogart, J. P. (1973) in *Evolutionary Biology of the Anurans*, ed. Vial, J. L. (U. Missouri Press, Columbia), pp. 337–349.