MEIOSIS: A SELECTION STAGE PRESERVING THE GENOME'S PATTERN OF ORGANIZATION

Lia Ettinger, Department of Biological Chemistry, Institute of Life Sciences, Hebrew University, Jerusalem, Israel

Received 7 March; 3 July 1986

ABSTRACT: The accumulating knowledge of recent years about the structure and organization of the eukaryotic genome, gives rise to new problems: what are the mechanisms that enable, during evolution, the frequent events of middle repetitive DNA reorganization without disrupting the whole function of the genome; what stops selfish DNA from accumulating in a genome, when the currently envisaged mechanisms for its propagation seem to be auto-catalytic in nature; why is the rate of divergence of most of the eukaryotic unique DNA slower than what is expected according to the "neutral theory" analysis? An attempt is made to analyse these problems from the wider perspective of two "classical" evolutionary problems: why is sexual reproduction beneficial, and the c-value paradox in eukaryotes.
It is proposed that meiosis serves as a selection stage, before the phenotypic level. This selection preserves the pattern of organization of the DNA in the chromosomes.
* * *

INTRODUCTION

Sexual reproduction is wide spread in nature. This well-known fact is, however, not trivial. It is commonly thought that sexual reproduction increases the genetic diversity within natural populations by reshuffling genes through meiosis and crossing-over, thus increasing the chance of some individuals to be better adapted to their environment. But the cost of meiosis is very high: each individual transmits to any of its offspring only 50% of its genes, giving an adaptive disadvantage of 50% (1). Thus, sexual reproduction must bear an enormous advantage if it is to be maintained in a population. Williams (1) describes a number of ecological circumstances in which the advantage of sexual reproduction may indeed be high. But according to Maynard-Smith's mathematical analysis (2), only very special types of selection patterns (which are not prevalent in nature) can account for the maintenance of sexual reproduction in a population which is capable of reproducing both sexually and asexually. Asexual reproduction is most prevalent in new vacant habitats and in unstable habitats like small temporary fresh water ponds (3). So it is unlikely that sexual reproduction provides opportunities for adaptations to novel and unpredictable future contingencies, as has often been claimed.
Since only about one in a thousand eukaryotic genetic systems is asexual (4), it seems to me, that the resolution of the enigma of the role and advantage of sexual reproduction should be sought by looking for basic processes shared by all eukaryotes. The key might lie in the differences between the eukaryotes and the prokaryotes and in the selective circumstances which brought about their divergence. For sexual reproduction is clearly most dominant among the former, while scarce among the latter.
Recent molecular analyses of the prokaryotic and eukaryotic genomes have revealed striking differences between them. Two major differences are the presence of introns and of large amounts of repetitive DNA, specific to the eukaryotic genome.
It might be useful to regard the divergence between the prokaryotes and the eukaryotes as a divergence between two modes of increasing efficiency in executing biological functions. The prokaryotic strategy is to increase efficiency by simplicity and economy. The eukaryotic strategy, on the other hand, is to increase efficiency by increasing complexity.
There seems to be a general trend of increasing DNA content with increasing complexity both among prokaryotes and among eukaryotes (5). In this case too, the prokaryotes and the eukaryotes differ substantially: in eukaryotes the correlation holds
* * * * * *

Evolutionary Theory 8: 17-26 (October, 1986)
The editors thank J.B. Walsh and another referee for help in evaluating this paper.

only for the minimum amount of DNA that is found in each phylum, otherwise there is no correlation between morphological complexity and the DNA content per cell and even closely related species may differ considerably in their genome size (6).
In prokaryotes, genome size does represent functional complexity (7). This leads to the question, why is this correlation kept among prokaryotes but not among eukaryotes. I want to show how this question is related to the problem of sexual reproduction. Or rather, how these two questions are linked with a third one, namely, how the size of the genome influences the selective pressure. I shall argue that the prokaryotes - eukaryotes divergence is connected with a critical genome size and the acquisition of superfluous DNA. Surpassing a threshold size enables superfluous DNA to accumulate, thus allowing the path of increasing complexity of the eukaryotes.
I shall elaborate the following arguments:
I first make a distinction between selectively neutral and adaptively neutral mutations. Claiming that under certain conditions natural selection cannot discriminate between different mutations that might be adaptively deleterious. I shall argue that most of the unique DNA is not adaptively neutral, and point to the fact that the large size of the unique component of the eukaryotic genome influences the intensity of selection, enabling adaptively diadvantageous mutations, including selfish DNA, to accumulate. Finally, I shall argue that meiosis serves as an additional selection step, before the phenotypic level, to prevent selfish DNA from penetrating functional parts of the genome.

Selectively neutral versus adaptively neutral mutations:

The debate between the selectionists and the neutralists has not yet been resolved. However, the dispute has shifted from the question whether neutral mutations exist to that of the relative frequency and importance of such mutations. Nucleotide substitution rates are especially high in DNA sites which are probably not subject to stringent selective constraints. The highest divergence rates are found in pseudogenes - DNA sequences which are homologous to known functional genes but have lost their ability to produce a functional product (8) - and in the third codon postion, where the code is often redundant (9). These findings clearly demonstrate that some mutations are indeed neutral.* By definition a neutral mutation hardly affects the fitness of the individual and therefore, its fate is determined by stochastic processes of genetic drift (9). Li (13) claims that a mutant can be regarded as effectively neutral, if its selection coefficient (s) - a measure of its differential reproductive success - does not exceed the reciprocal of the effective population size (Ne), ($|s| \leq 1/Ne$). In other words, the same mutation can be neutral in a small population and slightly deleterious in a big one. Thus the concept of selectively neutral mutations is a relative one.
It is important to make a clear distinction between adaptation and fitness. Fitness is the measure of the relative reproductive success of individuals in a particular population, at a particular point in time. Adaptation is a much less rigorously defined term which is used to explain fitness values. The Darwinian claim is that some individuals have a better chance to survive and reproduce, that is have a

****

*It is possible that the high rate of change in pseudogenes is a result of selection for divergence (i.e., to prevent recombination or transcription). So, the maximum drift rate calculated from pseudogenes data may be exaggerated. On the other hand, using rates of synonymous substitutions to calculate the maximum drift rate may give an underestimate, since codon usage bias is a common feature of most organisms and therefore imposes selective constraints on third base substitutions (10). The estimated rate of change in pseudogenes, however, is only about 1.9 times the rate for synonymous substitutions (11,12). Therefore, it is reasonable to assume that these rates give an adequate estimate for the order of magnitude of neutral substitution rates.

higher fitness, because they are better adapted to their physical and biological environment. It is very difficult to analyse adaptations, since each case of evolution is unique and because of the large number of variables in any biological system. Engineering optimization calculations (14) and efficiency in energy utilization (15) may be used cautiously to measure the level of adaptation. In spite of the difficulties, understanding adaptation is an essential part of understanding evolution. The measurement of fitness values enables us to compare different individuals or different alleles within a population, but it will give us no clue to the overall adaptation of the population, and will not allow comparisons between expanding and declining populations.

Although the level of adaptation is meaningful only in relation to a certain environment, it enables us to compare different populations and to predict which one has a better chance of survival. Despite the differences between adaptation and fitness, fitness values may be indicative of the level of adaptation of a trait. Comparing fitness values of a given mutation with the fitness values of its alleles over many generations, and a large range of environmental conditions, may indicate whether a mutation is adaptively neutral, since only mutations which have no adaptive value will remain selectively neutral for long periods of time. Therefore, it is relevant to ask in what situations will adaptively advantageous or deleterious mutations be selectively neutral. One example has already been mentioned, namely the case of slightly deleterious mutations which are selectively neutral in small populations. Another example is the case of trade-off between different mutations in different loci, in different individuals: different mutations may have identical fitness values in a particular generation and therefore their frequencies will not change relative to each other. Some deleterious mutations may be preserved in a population because of the balancing effect of other mutations at other loci, in other individuals: Deleterious mutations at other loci may decrease the fitness of other genotypes to the same extent that a particular mutation at a given locus does in a given genotype. Since selective pressure is proportional to the variance in fitness (16), the selective pressure exerted on a particular mutation is dependent on the number and character of other mutations in the same population.

King (17) argues, along the same lines, that selectively neutral alleles need not be without phenotypic effect. Artificial-selection experiments uncover ample underlying genetic variability for virtually any trait the experimenter may choose to select for. Therefore, he suggests that most molecular variations are phenotypically effective but, at the same time, molecular evolution is almost entirely due to random genetic drift. In populations of organisms with large genomes, each individual accumulates several new mutations at each generation, resulting in enormous genotypic variation in the population. But as the variance in fitness is generally relatively small (9,18), different individuals which represent different collections of mutations must have effectively identical fitness values. That is to say, the trade-off process is much more effective in populations of organisms with large genomes. From the point of view of natural selection many genotypes will be indistinguishable. But a mutation can be viewed as adaptively neutral only if it remains selectively neutral in many successive generations. However, this trade-off process is insignificant if most mutations were adaptively neutral. It will be argued in the next section that this is probably not the case.

Most of the unique DNA is probably not adaptively neutral:

Kimura (9) showed that if a large number of segregating loci or sites are involved in a quantitative character (in this case the overall fitness of the individual), the average selection coefficient per mutant under stabilizing selection must be exceedingly small. He concluded that in a mammalian genome consisting of 3.5×10^9 nucleotide sites, the majority of mutations at the molecular level do not affect the fitness of the individual. The attribution of selection coefficients to individual

alleles (and in this case to individual nucleotide sites) may be misleading. Sober and Lewontin (19) show that in many situations, especially in the case of quantitative characters, selection coefficients should be attributed to a higher level than that of the single allele (e.g. a pair of alleles, a gene complex, a genotype or a mating pair). In cases of adaptively neutral mutations, the selection coefficient of the allele in isolation, and its selection coefficient when in combination with other alleles at other loci is identical. This, however, is not the case for a selectively neutral mutation, because of the trade-off process which can occur in polygenic systems. Therefore, the only conclusion that can be drawn from Kimura's calculations is that during a single generation selection does not affect most DNA sites. The conclusion that these sites are adaptively neutral can be reached only if it is shown that they remain neutral over many generations in many genotypic combinations. Two lines of evidence suggest that a significant part of the eukaryotic genome is selectively meaningful: a) measurements of mutation rates both spontaneous and induced by chemical mutagens and by radiation. b) comparison of DNA sequences between species by DNA hybridization.

Spontaneous mutation rates per base-pair replication declined by at least one order of magnitude from Escherischia coli to Drosophila melanogaster (2). The sensitivity to mutagens, however, increases proportionally with genome size: i.e., double logarithmic plots exhibit a linear relation between genome size and mutation rate per locus per rad (20) or per amount of mutagen (21). The simplest explanation for this observation is that the target for mutation is roughly proportional to genome size. That is to say that the structural gene might be connected with more controlling DNA in organisms with large genomes (21). Molecular analyses in Drosophila indeed show that the size of the mutable gene is many times larger than the size of the functional transcript (22). The evidence that there was selection pressure for decreasing the rates of spontaneous mutations with increasing genome size (23), and that a correlation exists between mutagen target size and the genome size suggests that the evolutionary increase in genome size is selectively meaningful (23,24).

Comparison of DNA sequences between species can also be used to learn about the selective pressure exerted on DNA sequences throughout many generations. DNA hybridization experiments show that different single-copy regions of a given genome vary highly in the rate of inter-species divergence. Britten concluded that such comparisons leave little doubt that selective pressures retarded the rate of divergence of a majority of the single-copy DNAs (25). In a recent report (26) Britten presents a neutralist view diametrically opposed to that held by him in previous works (25,27,28). He claims that the median divergence rates calculated from DNA hybridization experiments of single-copy sequences between taxonomic groups are similar to the rates of divergence of neutral substitutions (synonymous substitutions). His conclusion that the majority of single-copy DNA is neutral might perhaps be drawn if the distribution of the fragments sampled by him represented a random sample of all the single-copy DNA sequences of the species examined. DNA hybridization experiments, however, give no information about the degree of divergence of the non-hybridizing (i.e., less conserved) sequences (27). Moreover, it is at least plausible to speculate that some of this divergence is due to selection that acted to give the species their unique identity. On the other hand, even in evolutionary distant species (species in which a large percentage of the DNA does not hybridize at all) there are still many highly conserved sequences - an observation which cannot be easily reconciled with the neutralist point of view (27).

This leads to the apparent contradiction that while, on the one hand, a considerable fraction of the unique DNA (which in mammals constitutes more than half of the genome (29)) appears to be conserved to some extent as the result of selection pressures, on the other (as Kimura (9) argues), there is insufficient variance in fitness for selection to affect most sites in the DNA.

This apparent contradiction stems from the confusion between adaptively neutral and selectively neutral mutations. Kimura calculates that if a neutral mutation becomes

fixed in a population, it takes on average 4xNe generations (four times the effective population size). But the average time of fixation of a selectively neutral mutation is not identical to the average time of fixation of an adaptively neutral mutation. Kimura's calculations hold only for adaptively neutral mutations, because the fate of these mutations is indeed only determined by genetic drift throughout the generations. However this is not the case for selectively neutral mutations which are adaptively deleterious. Their fate is determined in each generation by the distribution of other mutations at other loci in the population. In a sexually reproducing population mutations appear in different genetic combinations at different generations, so that a mutation may be selectively neutral in one combination and selectively deleterious in another. The above mentioned trade-off process decreases the chance of such mutations to become fixed. Therefore, if most of the genome is adaptively meaningful, most DNA sites will be substituted less frequently than adaptively neutral sites like pseudogenes and third codon positions. The evidence cited above indicates that this is probably the case.

Selfish DNA in eukaryotes versus prokaryotes:

Doolittle & Sapienza (30) and Orgel & Crick (31) suggested that "selfish DNA" may account for most of the superfluous DNA in eukaryotic cells and explain the c-value paradox. They defined "selfish DNA" as DNA sequences within the genome that do not contribute to the phenotype, but may reproduce faster than the rest of the genome, thus spreading in many copies within the genome. The amount of selfish DNA sustained by a genome could be determined by an equilibrium between selective pressures between genomes and within the genome.

It may be hard to prove the existence of selfish DNA, but clearly the eukaryotic genome has the potential to generate it, considering the large amount of repetitive sequences and transposon-like elements in the genome. Dover (32) points out that the eukaryotic genome is very fluid, as a result of multiple events of unequal exchange, gene conversion and transposition. Repetitive sequences structured like transposons have been discovered in many species, among them: yeast, nematode, Drosphila, maize, mouse and man (33). The presence of pseudogenes suggests that new sequences of DNA can be formed on a RNA template and be integrated into the genome (8). A large fraction of the mammalian DNA may have originated as RNA that was written back into DNA. Reanney (34) calculated that there has been a reintegration event in the germline for about 10% of the genes within the last 10-20 million years.

Whatever might be the evolutionary origin of the dynamic eukaryotic genome, with its repetitive sequences and introns, it seems plausible that these characteristics enabled the eukaryotes to take the path of increasing complexity. Superfluous DNA so generated may acquire new functions with time. On the other hand, superfluous DNA might disrupt gene action. It is obvious that processes such as unequal exchange and gene conversion can affect gene action.

There is direct evidence that many Drosophila mutations result from the insertion of copia-like elements (35). Crick and Orgel (31) attribute all the deleterious effects of selfish DNA to its metabolic cost and claim that this cost is relatively low. But a more central role should perhaps be attributed to the need of a dynamic and unstable eukaryotic genome to protect itself from the disruption of genes and of control sequences brought about by the above mentioned processes. In prokaryotes, each such change is immediately tested by selection. This is possible because the variation in genotypes in each generation is small enough to be represented in selectively meaningful fitness differences. But as is argued above, selection cannot effectively eliminate all the mutations that arise in organisms with large genomes. Therefore some selfish DNA may accumulate even when it is adaptively disadvantagous, because its effect will be below the threshold of selection in a complex genotype. The diploid state of most eukaryotes also reduces the rate of elimination of disadvantagous mutations because it permits the accumulation of recessive mutations.

Moreover, selfish DNA differs in its chances to survive from that of ordinary mutations, because the process of its accumulation is self perpetuating: the more repetitive DNA and transposons accumulate in a genome, the higher the chance of transpositions and unequal crossing-over events. Also, because in prokaryotes most of the DNA is functional, it is most likely that each transposition event will cause a mutation that will probably be eliminated by selection. When selfish DNA accumulates in a eukaryotic genome, however, it also forms neutral sites for further selfish DNA integration.
Because on the one hand selfish DNA is self-propagating, and on the other hand due to the trade-off process phenotypic selection is ineffective against selfish DNA, it may accumulate in the genome. The phenomenon of hybrid dysgenesis indicates that the mutabiltiy potential of transposons in the germ line is enormous. The dysgenic progeny carry a very large number of visible and lethal mutations (36). This potential is,however, not often expressed. The large differences in repetitive DNA between related eukaryotic species both in sequence order and in the distribution within the genome (37,38) imply that rapid organizational changes of DNA sequences occur frequently during evolution. How can such reorganization occur without causing major damage similar to that caused by hybrid dysgenesis? The frequent occurrence of such reorganization events implies that in organisms with large genomes, there must be an additional mechanism to prevent selfish DNA from disrupting gene functions.

Meiosis as a selection step:

It has been suggested that the main function of meiosis is DNA repair of damage which occurs simultaneously on both strands of the DNA (39). Holliday (40) proposed that the removal of epigenetic defects by recombination is an essential function of meiosis. He also suggested that selfish DNA insertion loops may be excised during the pairing process at meiosis (41). I want to suggest that the process of meiosis in organisms with genomes above a critical size may have acquired an additional function: that of maintaining an overall organization pattern of the genome. A maintenance of such an organization can prevent the spreading of selfish DNA into functional regions, and may prevent phenomena similar to hybrid dysgenesis.
It seems useful to compare selfish DNA with gene mutation. Both may confer a selective advantage upon an organism yet both may be hazardous. Mutation repair mechanisms are usually very efficient, i.e. most mutations do not survive to go through processes of natural selection. But some mutations do escape repair and have the opportunity to be tested by selective processes. I would like to propose that there is also a "repair mechanism" to eliminate superfluous DNA - and that this mechanism is not 100% efficient, thus enabling some of the superfluous DNA to survive, and possibly to become functional.
In order to get rid of selfish DNA, there must be a mechanism for identifying it as such. A simple solution would use a template based tester. Hypothetically, such a template does exist - in any diploid cell each chromosome can act during meiosis as a template for its homologue. The template tester should not be too stringent, since the presence of different alleles in the two homologues implies a certain amount of variety. There are some families of middle repetitive sequences, such as P-DNA in Lilium that are highly conserved in evolution (42). The P-DNAs account for 1% of the genome, and are labeled during the later phase of pachyten as a result of repair mechanisms. It is possible that crossing-over events in these targets enable to check for perfect alignment between the homologues. Thus if selfish DNA penetrates a region that is defined by the pattern of middle repetitive sequences as vital, it will trigger a process that would result in the death of the aberrant gamete.
This postulated mechanism implies a high degree of order in eukaryotic DNA. Selfish DNA can be sustained by a genome only if it integrates without disrupting the inbuilt pattern of the genome. Indeed, several studies imply that although related species may have very different repetitive sequences, a certain pattern of organization of

the repetitive sequences is conserved (37,38). Lima-de-Faria's work on the chromosome field also implies a high degree of conserved order in the eukaryotic chromosome (43).

Transposition events that escape the selection process may lead to phenomena similar to hybrid dysgenesis and may result in a partial reproductive barrier between two sub-populations and therefore bring about speciation (44,45). Dover suggested that molecular drive is a common mechanism for speciation (32).

To summarize, meiosis may serve as the sentinel of the big, dynamic eukaryotic genome. Failure of the meiotic selection stage would usually lead to extinction. On rare occasions, however, it may lead to speciation.

To test the meiosis-repair hypothesis, one should try to compare the amount of change per generation in the genome of somatic cells with that of germ cells. The excision and insertion rates of transposable elments in somatic cells may give some measure of the degree of fluidity of the genome. According to the suggested model, transposable elements can survive in the germ-line only if they adapt themselves to the inbuilt pattern of the genome which will enable them to survive during meiosis. Therefore it suggests that the mobility of transposons in the germ line will be very limited compared to that of somatic cells. Recently, Emmons and Yesner (46), published a report that in the nematode Caenorhabditis elegans the excision of a transposable element seems to be confined to somatic tissues. They found a high rate of excision in somatic cells (around 10% per generation) while the germ-line excision events, if they occur at all, occurred at a frequency much less than 1% per generation. This excision might be connected with developmental regulation, or else controlled by factors that distinguish the somatic cells as a whole from the germ cells. In many invertebrates, however, excision of the gonads does not sterilize the animal since the adjacent somatic tissues may regenerate to form new gonads (47). It is possible that it is the special cytoplasm of the germ-cells that gives them extra protection from the environment. But, since the processes of unequal exchange, gene conversion and transposition are common to all living organisms, and all types of cells, it is hard to envisage the germ cells to be different in that respect. I suggest that the uniqueness of the germ cell lies not in a different kind of cytoplasm, but in meiosis which serves as a selection stage against the accumulation of germ cells with altered DNA.

Transposons may evolve mechanisms for the regulation of their own transposition. Transposons replicating in the soma do not contribute to the next generation and at the same time may cause damage to the host and to their own propagation. Selective advantage will, therefore, be conferred upon transposons which can regulate their own transposition in the soma (48). Charlesworth and Langley (48) also discuss the conditions that may lead to the evolution of self-regulation of transposition in the germ-line. They concluded that such regulation will tend to evolve only in organisms with small genomes and restricted recombination. Transposition-repression will evolve in organisms with free recombination only if the frequency of immediately-expressed lethal or sterile mutations is sufficiently high, or if coupling of regulatory effects exists between different transposon families. In the latter case a mutation in a member of one family of transposons affects the rate of transposition of several other families. Such coupling has not as yet been demonstrated. The model presented in this paper, however, suggests a different kind of coupling mechanism - transposition events of all transposable families are selected against during meiosis. It is, therefore, reasonable to assume that selection pressure for the evolution of such a general mechanism will be high, even if the mechanism is only partially successful.

Some implications of the genome repair model of meiosis:

Some features of oogenesis and spermatogenesis may support the model of meiosis as a genomic selection stage. In mammals, sperm and eggs are generated in large

quantities, but many of these cells are eliminated by degenerative processes. These processes are not confined to mammals. Similar degenerative processes have been observed in a wide range of organisms. For example: lampreys (in females of Petromyzon marinus only 20-30% of the oocytes survive the atresia process (49)), grasshoppers Melanoplus differentialis (50), Xenopus laevis (51) and chickens (52). In oogenesis of mammals a few million egg cells are formed, but only a small number reach maturity - the rest are eliminated by the process of atresia. In spermatogenesis 27-37% of the sperm cells are eliminated during the mitotic and the meiotic divisions (53). These processes seem very wasteful, but they may be vital if they represent selection on the level of the germ line population. It has been shown that exposure of the ovaries and the testes to ionizing radiation increases the process of atresia and that damaged cells are specifically eliminated (53). It is not clear what makes one egg follicle mature, while the follicles around it remain unaffected. It has been suggested that the signal for maturation comes from the egg cell itself (54). I suggest that the signal arises from the chromosomal state of the egg cell. The egg cells in the ovaries, awaiting maturation, are arrested at the dictyotene stage of the first meiotic division. These chromosomes have a very diffuse morphology, and although they may be arrested at this stage for years, they show a high rate of metabolic activity. It seems possible that, at some time during this stage, specific sequences of the two homologous chromosomes are compared, and only cells which exhibit a good measure of homology are allowed to complete the meiotic cycle. Williams states that that "while it may be difficult to identify any species for which it can be confidently asserted that exclusively sexual reproduction is selectively advantageous, it is very easy to identify some in which it must be disadvantageous. All organisms with really low ZZI [potential zygote-to-zygote increase] such as mammals, birds and many insects have populations in which asexual reproduction must be consistently selected against" (55). The model of meiosis as a genomic selection step seems especially appropriate for such low fecundity organisms. The prediction of the proposed model is that selective processes during meiosis will be prevalent among eukaryotes and essential among low fecundity organisms with a genome above a critical size.

Sexual reproduction is common even among unicellular eukaryotes. Many species of protozoa exhibit a finite growth potential and fertilization leads to rejuvenation - resetting the age clock to zero (56). The more complex the organism and the more it invests in its offspring, the more crucial, however, are the requirements for repair and selection mechanisms. Thus it seems that the the greater impetus for refinement of sexual reproduction arose with the evolution of multicellularity from a single cell.

ACKNOWLEDGEMENTS

I would like to express my gratitude to Professors D. Cohen, R. Falk and W. Stein of the Hebrew University, for their comments and advice. Special thanks are due to my friends and colleagues Debora Kamrat and Eva Jablonka for continuous critical discussion and encouragement. I would also like to thank the referees for their helpful suggestions.

REFERENCES

1. Williams, G.C. (1975) "Sex and Evolution", Princeton University Press.
2. Maynard-Smith, J. (1978) "The Evolution of Sex", Cambridge University Press.
3. Bell, G. (1982) "The Masterpiece of Nature: The Evolution and Genetics of Sexuality", University of California Press, Berkeley.
4. White, M. (1978) "Modes of Speciation", W.H. Freeman and Company, p. 315.
5. Sparrow, A.H., Price, H.J. and Underbrink, A.G. (1972) A survey of DNA content per cell and per chromosome of prokaryotic and eukaryotic organisms: some evolu-

tionary considerations. In: "Evolution of Genetic Systems", Brookhaven Symposia in Biology No. 23 (ed. Smith, H.H.), Gordon and Breach, New York, pp. 451-494.
6. Cavalier-Smith, T. (1978) Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. J. Cell Sci. 34: 247-278.
7. De Ley, J. (1968) Molecular biology and bacterial phylogeny. Evol. Biol. 2: 103-156.
8. Proudfoot, N. (1980) Psuedogenes. Nature, Lond. 286: 840-841.
9. Kimura, M. (1983) "The Neutral Theory of Molecular Evolution". Cambridge University Press.
10. Berger, E.M. (1977) Are synonymous mutations adaptively neutral? Am. Nat. 111: 606-607.
11. Miyata, T. and Yasunaga, T. (1981) Rapidly evolving mouse α-globin-related pseudo-gene and its evolutionary history. Proc. Natl. Acad. Sci. USA 78: 450-453.
12. Li, W.-H., Gojobori, T., Nei, M. (1981) Pseudogenes as a paradigm of neutral evolution. Nature, Lond. 292: 237-239.
13. Li, W.-H. (1978) Maintenance of genetic variability under the joint effect of mutation, selection and random drift. Genetics 90: 349-382.
14. Lewontin, R.C. (1978) Adaptation. Sci. Am. 239: 213-220.
15. Peters, D.S. (1983) Evolutionary theory and its consequences for the concept of adaptation. In: "Dimensions of Darwinism" (ed. Grene, M.), Cambridge University Press, pp. 315-327.
16. Fisher, R.A. (1958) "The Genetical Theory of Natural Selection", Dover Publ. Inc. New York.
17. King, J.L. (1984) Selectively neutral alleles with significant phenotypic effects: a steady-state model. Evol. Theory 7: 73-79.
18. Lewontin, R.C. (1974) "The Genetic Basis of Evolutionary Change". Columbia University Press, New York.
19. Sober, E. and Lewontin, R.C. (1982) Artifact cause and genic selection. Phil. Sci. 49: 157-180.
20. Abrahamson, S., Bender, M.A., Conger, A.D. and Wolff, S. (1973) Uniformity of radiation-induced mutation rates among different species. Nature, Lond. 245: 460-462.
21. Heddle, J.A., Athanasion, K. (1975) Mutation rate, genome size and their relation to the rec concept. Nature, Lond. 258: 359-361.
22. Poole, S., Kauvar, L.M., Drees, B. and Kornberg, T. (1985) The engrailed locus in Drosophila: structural analysis of an embryonic transcript. Cell 40: 37-43.
23. Auerbach, C. (1976) "Mutation Research" Chapman and Hall, London.
24. Cavalier-Smith, T. (1985) Eukaryote gene numbers, non-coding DNA and genome size, in: "The Evolution of Genome Size" (ed. Cavalier-Smith, T.), John Wiley & Sons, pp. 69-103.
25. Britten, R.J. (1982) Genomic alterations in evolution. In: "Evolution and Development" (ed. Bonner, J.T.), Dahlem Konferenzen, pp. 41-64.
26. Britten, R.J. (1986) Rates of DNA sequence evolution differ between taxonomic groups. Science 231: 1393-1398.
27. Hall, T.J., Grula, J.W., Davidson, E.H. and Britten, R.J. (1980) Evolution of sea urchin non-repetitive DNA. J. Mol. Evol. 95-110.
28. Grula, J.W., Hall, T.J., Hunt, J.A., Giugni, T.D., Graham, G.J., Davidson, E.H. and Britten, R.J. (1982) Sea urchin DNA sequence variation and reduced inter-species differences of the less variable DNA sequences. Evolution 36: 665-676.
29. Lewin, B. (1983) "Genes", John Wiley & Sons.
30. Doolittle, W.F. and Sapienza, C. (1980) Selfish genes, the phenotype paradigm and genome evolution. Nature, Lond. 282: 601-603.
31. Orgel, L.E. and Crick, F.H.C. (1980) Selfish DNA: the ultimate parasite. Nature, Lond. 282: 604-607.