EVOLUTION BY MUTATION[1]

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It is not possible for me to represent the high tradition of Josiah Willard Gibbs by offering you a mathematical treatment. Nevertheless, the subject of biological evolution and its mechanism must be of great interest to yourselves, as the most exemplary products of its operation. Perhaps, then, our reconnaissance flight over these biological jungles, and our attempts to measure certain aspects of them, may serve to entice some of you or, through you, some of those with whom your influence counts, into bringing your higher powered mental tools to bear in the more effective and more elegant mapping and analysis of this territory. If so, my intention to inveigle you into it will have been successfully accomplished.

To those philosophers who declare "I think, therefore I am," their own existence seems the one complete certainty. To others, it does not seem so certain that they do think, nor even that they produce a significant imprint on reality in general. It is, however, evident that they, along with all things living, if they do exist, are utter improbabilities, far less plausible than any other phenomena that have been encountered.

Herein we shall attempt to assess how fantastically unlikely we and our fellow creatures are, and by what means such preposterous anomalies could have come about. The old-time philosopher still insists that such extravagances of organization could have arisen only by design, inasmuch as accident cannot be expected to convert itself into order. However, a dispassionate examination of the rules of this game of life should throw some light on the question of how such a massive compounding of improbabilities may have taken place.

1. **The genetic alphabet.** Studies in Mendelian heredity, supplemented by microscopic observations, gave evidence some half century ago that at the core of our being, and of that of every living thing, there is a remarkable material, that is particulate, exceedingly constant in its parts, subject to orderly mosaic rearrangements, and in a sense self-multiplying. All this was shown by the kaleidoscopic, yet statistically predictable effects it gave on being transmitted and

The thirty-first Josiah Willard Gibbs Lecture, delivered at Cincinnati, Ohio, on January 28, 1958, under the auspices of the American Mathematical Society; received by the editors March 14, 1958.

[1] This article is Contribution No. 649 of the Department of Zoology, Indiana University.

multiplied from generation to generation in the form of diverse combinations. The term "genes" was applied to the regularly recombining parts of this mosaic. Moreover, the fact was established on the basis of the quantitative relations which were observed in the recombinations of these genes, that they are strung together in a single-file arrangement, like the links in a chain, so as to form the microscopically visible filaments called chromosomes [1].

It further became clear that despite the constancy of the individual genes they are separately subject to rare, sudden changes, or "mutations," from one stable state to another. This is proved by the changed effects on the descendants that inherit them after such an occurrence. For these descendants then constitute exceptions to the original predictions. They are, potentially, the seed of new, although usually only slightly new, forms of life.

The most unique characteristic of these genes has long been realized to lie in the fact that, after a mutation has occurred, the gene in its changed form, on reproducing, gives rise to daughter genes that incorporate its new feature. That is, the mechanism of the gene's self-reproduction is such as to result in the perpetuation and, if circumstances permit, the multiplication of the deviant type. As has been pointed out elsewhere [2], it is the possession by the gene of this faculty of self-copying, of a kind that is capable of being retained despite changes in that gene's own composition, that causes the gene to serve as the basis of evolution. And the enormous complications to which evolution may go are made possible by the fact that these changes in genes and in groups of genes can become accumulated to a virtually unlimited extent, without entailing the loss of the genes' self-copying faculty. Moreover, it has become clear that, as had been surmised, the self-copying involves an attachment, next to each characteristic component of the gene, of a particle of corresponding[2] type that had been floating about in the medium surrounding the gene. In this way there becomes pieced together next to each gene a replica of itself, that is, a structure having the same internal pattern. A mutation consists of a permutation in this pattern.

Through the brilliant recent theory of Watson and Crick [3], backed by strong evidence from work of Benzer, Hershey, Stanley and many others, it has been virtually proved that the components in question are nucleotides, combinations of phosphoric acid, a simple

[2] As will be seen in what follows, however, the "corresponding" type here turns out to be a complementary one, rather than an identical one as had been first assumed. But since the gene contains pairs of complements to begin with this process works out to give a product that is identical with its producer after all.

sugar, and a nitrogenous base, the whole having a molecular weight of about 300. In the gene there are only 4 types of these nucleotides, that we may here call *A*, *B*, *C* and *D*. The gene consists basically of these nucleotides polymerized into the form (termed DNA) of a pair of relatively long parallel but coiled chains, of which the nucleotides form the links. In any such pair of chains *A* is always complementary to *B* and *C* to *D*, in such a way that *A* in one chain regularly has *B* lying opposite to it in the other chain, and *C* has *D* opposite to it. These opposite, or rather, complementary components form effective cross-unions with one another, and not with the other types of nucleotides, by means of hydrogen bonds. It is this fact that explains their selectivity in attaching to themselves only appropriate (complementary) particles derived from the medium, during the process of gene reproduction.

Now, although there are only two types of nucleotide-*pairs*, these amount to four types so far as the gene is concerned. For their arrangement within the gene is different according to which of the two members of a pair of nucleotides lies in a given member of the pair of chains. Hence, unless there are additional features that we do not yet know about, we could specify the entire composition of a gene through the use of four letters, *A* to *D*, setting them down in line, as in a word, in the order in which they occur in either one of the two members of the double chain. As yet, we are far from knowing this order in any case. But there is reason to infer that a gene-word is composed of thousands, even tens of thousands, of "letters."

A mutation, on this scheme of representation, consists in the substitution, loss, or insertion, of one or more of these same letters. Benzer's work [4] may be taken as indicating that only one letter, or nucleotide pair, is usually involved, but that at times a whole block of them may be inverted *in situ*, lost, or inserted. This same principle of what may be called point and line mutation has long been known to hold, on a far larger scale of magnitude, in the case of those greater chains, the chromosomes, the links of which are whole genes, some hundreds or thousands of them per chromosome.

2. **A measure of our own improbability.** We are now in a position to make some first estimates of the degree of improbability represented by our own genetic material. The total mass of nucleotide material, or DNA, contained in one set of human chromosomes, such as would be found in a human sperm or egg nucleus just before they united in fertilization, is approximately 4×10^{-12} of a gram. Since the mass of one pair of nucleotides is about 10^{-21} of a gram there

must therefore be about 4×10^9 nucleotide pairs in the chromosome set.

It is not certain that in higher animals the gene string, as we call it, contains only *one* double chain of nucleotides, but there is morphological as well as autoradiographic evidence that this is the case in bacterial viruses, and autoradiographic evidence in some higher plant material also. Moreover, the way that mutant genes have been observed to express themselves in some higher forms after a mutation has occurred, that is, the fact that in some cases all, in some cases about half of the cells descended from the cell in which the mutation has occurred may receive a replica of the mutant gene, indicates that this gene had not been in the form of more than two parallel threads. It therefore appears highly probable that even in man the genetic material of the sperm cell is in the form of unreplicated, merely double, chains of nucleotides.

This would lead us to conclude that all human gene strings of one chromosome set taken together contain some 4×10^9 nucleotide pairs arranged in one double line. It is possible some of the nucleotides are not in this line and are nongenetic, as Levinthal's preliminary results on bacterial viruses [5] had indicated to be the case in them. However, certain more recent findings have raised questions concerning this interpretation in the viruses, and the higher plant studies by Taylor et al. [6] have given grounds for considering virtually all the chromosomal DNA in them to be genetic.[3] This is a matter that the application of autoradiography to higher forms should soon give definite information about. Meanwhile, it will here be assumed that the number of genetic nucleotide pairs arranged linearly in a human chromosome set is the full number, 4×10^9, present in a human sperm cell.

Inasmuch as for each nucleotide pair there is a choice of 4 possible forms (representable as *A*, *B*, *C* or *D*) in a given member of the double chain, it is evident that the number of possible permutations of these four forms, in a line containing four billion of them, is four to the four billionth power or approximately $10^{2,400,000,000}$. It is true that this number should be reduced by dividing it by the number of permutations that would be possible among the 23 chromosomes and, more important, among all (some 10,000 to 40,000) entire genes, on the dubious supposition that most of these permutations would leave the genes' effects substantially unchanged. Nevertheless, on

[3] At the time of the lecture the reports giving the most recent evidence had not yet come to hand and the conservative assumption was therefore made that in man only 40% of the chromosomal DNA is genetic. Thus, in the text that follows, the figures are correspondingly higher than those that were presented orally.

making the maximum possible estimate for the magnitude of this divisor, a "mere" $10^{270,000}$ at most, we find the size of our exponent reduced by an amount that is entirely insignificant, in terms relative to its own size, and we may therefore feel justified in settling on the above approximation. Now, since any given individual chromosome set represents but one combination we may say that the "chance" of its occurrence is the reciprocal of this number, or $10^{-2,400,000,000}$.

It should be recognized that this figure may give an exaggerated impression of our uniqueness, since we do not know whether many nucleotide substitutions might be made that would have no effect, or virtually none, on the resulting organism. Moreover, many of them have such relatively slight effects as we see differentiating the persons about us. As against this consideration, however, there are grounds for inferring that losses of nucleotides, or of blocks of them, from the chain, occur as much more frequent accidents than do gains (that is, insertions) of them, so that there is a tendency for unnecessary elements to be eliminated eventually. Let us then take our approximation at its face value and try to arrive at a working idea of its magnitude by comparison with something familiar to us in everyday life. It can be estimated that a large, finely printed edition of Webster's Unabridged Dictionary contains about thirty million letters. If, then, we used only the letters *A*, *B*, *C* and *D*, to represent the four nucleotide pairs, we could represent the entire arrangement of them in a single human sperm or (pre-fertilization) egg nucleus by the use of about 133 volumes, each of the size and fineness of print of this dictionary.

Here, presumably, we should have the entire genetic specification for a man, at least so far as his inheritance from one of his parents was concerned, and another 133 volumes would give that from his other parent. With the know-how (as yet not in sight) of how to string nucleotides together indefinitely as desired, and to give them the right wrappings, we should then be able to insert them into an egg from which its own nucleus had previously been removed and thus, after enormous labor, helped perhaps by automation, to produce a man as much like the one who had furnished the specifications as if he were an identical twin. Or we might incorporate alterations in him to order.

If instead of representing each nucleotide-pair separately by *A*, *B*, *C* or *D*, we utilized the entire English alphabet of 26 letters, plus half a dozen distinctive Cyrillic letters to make 32, and if we then allowed the letters to be either in lower case or capitalized, either in the slanting italic or vertical roman style, and either heavy faced or fine, thus gaining 256 distinguishable characters, we could allow each character

to represent a group of four nucleotide pairs instead of just one pair (since for each group of 4 there would be 256 possibilities). We could thereby reduce our 133 volumes to 33. We could also greatly condense the representation of the inheritance from the second parent by designating only those items of it that differed from the corresponding items derived from the first parent, and by inserting these modifications, with appropriate punctuation, at the points in question, in line with the specifications of the first parent's contributions. By then transferring our perhaps 34 great volumes to especially thin microfilm we should be able to get our coded *homo* into the space of one volume having the outer dimensions of a scientific handbook. However, we may recall that, by contrast, the actual nucleotide material of a human or other mammalian germ cell, when mature, would occupy only about four cubic microns, of weight 4×10^{-12} grams.

3. **An alternative measure.** There is an older method of estimating our improbability [7] that can now be brought more nearly up to date. Both observations and general considerations make it likely that, very conservatively, not one among 100 mutational changes with a presently detectible effect on the organism is conducive to its survival or fertility and thereby favors the multiplication and establishment in the species of the given mutant type. Moreover, any accidental accumulation of smaller changes that together resulted in as much deviation from the original type as those here in question would have a similarly small chance of being advantageous. The reason for this prevailingly detrimental character of mutations is of course the fact that there are far more ways of damaging the workings of an already elaborate and well constructed organization than there are of improving it even further. This situation is analogous to that of the second law of thermodynamics. In the latter case the energy of particles subject to random motion tends to become dissipated because of the fact that there are more directions and amplitudes of movement by which the energy can be scattered than those by which it can be concentrated. So, in general, there are more types and degrees of change that are disorganizing in relation to the production of a specific result (in the case of living things, their multiplication) than those that are further organizing.

Simplifying the situation by first considering only nonsexually reproducing organisms, and taking the conservative estimate of 99 detrimental to 1 advantageous change of perceptible degree, it follows that, on the average, the mutant type must multiply at least a hundred fold after each advantageous mutation if evolution is to

continue. This multiplication is necessary to make the individuals, or rather, the lines of descent, of the advantageous type numerous enough to allow just one of these hundred lines to give rise to a second advantageous mutation, added to the first one. And so on after that, for each successive advantageous mutation that is accumulated in the same line of descent, there must on the average be a further multiplication of at least a hundred times.

In the meantime, the individuals with the disadvantageous mutations, and in some cases those of the original type also, will have tended to die out, thus making room for the line having the concentration of favorable changes. For the latter, however, the rate of multiplication will have averaged so high that, had this same rate characterized the entire population, their final number would have been at least 100 to a power equal to the number of successive advantageous mutations that accumulated in the favored line. Thus, for 3 beneficial mutations this number would have been 100^3 (or 10^6) and for 100 mutations, 100^{100} (or 10^{200}).

These figures are relevant to our inquiry into the degree of our own improbability. For suppose that, instead of having started with just one individual which, in its more favored lines of descent at least, was able to multiply at a rate that would have given the number calculated, we had, instead, *started* with as many individuals, and therefore with as many lines of descent, as that imaginary final number that would have resulted from the equal multiplication of all lines at a rate as high as that of the favored lines. There need in that case have been no multiplication at all, or any ability to multiply, but only a persistence of the individuals, or of their single-file "lines of descent." In fact, even this persistence need have occurred only in the lines in which successive "favorable" changes happened to occur. Yet, given the same rate of "mutation" as before, we should on this system have ended up with just as many individuals having the maximum number of "favorable" changes as on the other system. For in both cases an equal number of individuals would have been provided, in each generation, in which disadvantageous (i.e. self-eliminating) mutations had not yet occurred, and in which "favorable" ones (i.e. those of types analogous to the mutations which would have favored multiplication had it been possible) could therefore have accumulated instead.

4. Differential multiplication as the extractor of the improbable. This "thought experiment" (to use the physicists' term) is, like most such experiments, fantastic, but illustrative of a principle. In this

case it shows not only the degree of improbability achieved by the succession of mutations in the favored lines but also the role played by the process of biological multiplication in allowing this degree of improbability to be achieved. Thus the scheme on which there was no multiplication shows that the individual that had accumulated 100 favorable mutations represented a combination of chances that could happen only once in 10^{200} trials. There is no possibility that this number of trials could ever, on our earth, have been achieved. Yet the process of multiplication, by being differential, that is, largely confined to the lines that continued, accidentally, to have the favorable mutations in them, succeeded in providing the opportunity for the realization of this degree of improbability.

Within the narrow confines of our world, this multiplication of the favored lines was able to occur only because space was left by the dying out of the other lines. That, then, was the role of selective elimination: to make room. But advantage could be taken of that room only by reason of the gene's faculty of reproducing itself, and thereby multiplying. And even this could not have resulted in evolution if the gene had not been so constituted as to reproduce its mutational changes also, in the process of reproducing itself. Evolutionary adaptation is thus the automatic result of the differential multiplication of mutations. And living things are so much more elaborately organized than nonliving ones because the gene's unique property of self-copying constitutes the basis for this differential multiplication of its changes.

Thus, on the primitive earth, after the myriad interactions of diverse substances, occurring in a medium of water and powered by high-potential discharges of photons and electrons, had resulted in the production of nucleotide molecules, and then attachments between some of them to form naked genes of the most rudimentary type, that fed on those that were free, their further evolution to produce their protoplasmic wrappings and finally all the complications of the intricately adapted organisms of today, followed from the pressure of their differentially multiplying mutations.

But we have not yet followed far enough in applying this method of estimating the degree of our improbability. This method, it may be recalled, proceeds by first estimating, conservatively, the probability that a given mutational step will be successful, and it then raises this figure to a power equal to the estimated number of such steps.

On reconsideration of the probability of success, which is the reciprocal of the number of mutations necessary, on the average, to

include one that is successful, it might at first sight seem that, for a given nucleotide-pair, the number of possible substitutional changes that would include one successful one should be no more than 3, since there are only 4 types among which to choose and one of these four types is already present. This inference, however, besides overlooking possible losses, insertions, and inversions, neglects the much more important fact that on the great majority of occasions any change at the given point would be disadvantageous. Usually there would be no possibility of any change at some *given* point in the nucleotide chain being advantageous until some change or combination of changes, of given kinds, had occurred elsewhere, that somehow upset previously attained adaptations. In other words, a group of successful steps accumulated over a period that, in terms of evolutionary time, is very long, would not have been successful if they had arisen in a radically different sequence from the actual one. This restriction explains why the chance of success for a change occurring at any given point, at any given time, can be less than 1 in 100 or even less than 1 in 10,000 despite the fact that the number of possible changes at that point is (if we exclude the comparatively rare cases of insertions and inversions) very limited.

Accepting, then, the very conservative figure of 1 in 100 for the chance of a given mutation being successful, what shall we assume for the exponent of this figure, that is, for the total number of successful mutations in the ancestry of a given higher organism? This total number may obviously be regarded as the product obtained by multiplying the number of successful mutations that have occurred per gene by the total number of genes. As for the number of past mutations per gene it is to be observed that, as was realized long ago, e.g. [7], each individual gene must be highly adapted and complicated, and have arrived at its present form through numerous steps. Knowing, today, that it contains thousands or tens of thousands of nucleotide pairs, we might estimate the number of steps per gene to have been as great as this or even much greater; that is, we might assume a past history of several or many substitutions of each pair. Nevertheless, we are, to remain on the conservative side, contenting ourselves at this point with the undoubtedly far too low figure of only 100 successful steps per gene.

In taking this figure we are bearing in mind the fact that in the distant past the genes were derived from one another, through rare accidents such as occasionally happen even today, whereby a block of them derived from one chain becomes inserted at some point into another chain. In consequence, many of the earlier mutational steps

occurred in genes that were common ancestors of several or many present-day genes, and our assumed number of 100 steps per gene refers to the total number of independently arisen mutations, averaged out per gene. Yet even considering this, the actual number of steps is more likely to have been many thousands than only 100 per gene, because there are grounds for inferring that gene numbers of the order of those at present existing were already attained more than half a billion years ago.

Taking now the number 10,000, derived from flies, as a minimum estimate for the number of different genes in a higher organism (despite the fact that the higher organism contains a far larger total number of nucleotides), we see that there must have been at least $100 \times 10{,}000$, that is, a million separate successful mutations in the ancestry. Applying this million as an exponent to 100 (our conservative figure for the reciprocal of the probability that a mutation will be advantageous) we then get $100^{1,000,000}$ (or $10^{2,000,000}$) as the total number of trials that would have been necessary, in the absence of multiplication and selection, to obtain one combination as well organized as our own or as that of some other advanced organism.

Although so much smaller than our other estimate of about $10^{2,400,000,000}$, based on the number of nucleotide pairs, the present more conservative number deserves some scrutiny, some comparison with more familiar things. In this connection we may ask, how much room would it have taken to contain this many combinations of genes at one time, in order that amongst them our own constitution might find a place as one of these random occurrences? A sphere having a diameter of six billion light years goes far beyond the most distant galaxies now detectable. For our present purposes, however, we shall call it, by a stretch of terminology, "the known universe." A little arithmetic will show that in this vast expanse there would be room, if they were all packed closely together, for about 6.25×10^{100} packets or skeins of nucleotide chains, such that each skein contained as many nucleotides as we have taken to exist in a mammalian sperm nucleus, namely, 4,000,000,000, the number that we previously found it necessary to employ 133 Webster's volumes to represent. Yet we see that this enormous number of packets, 6.25×10^{100}, is inordinately smaller than the number $10^{2,000,000}$, that on our more conservative estimate could be expected, as a random event, to include a packet with a composition as select as our own. And even if we had some science-fictionist's method of reducing the size of a genetic packet to that of a proton, we could still get only about 10^{128} of them into the known universe.

Suppose, now, that in order to attain our desired number we allowed each of these packets or genetic combinations to exist for only a millimicrosecond, that is, a billionth of a second, and then caused it to be replaced by a different combination, and so on every millimicrosecond in succession for six billion years, which is probably longer than the earth has existed. This would have allowed some 2×10^{26} changes and we should thereby be able to accommodate in the "known universe" during this period about 2×10^{154} genetic combinations. Let us institute next the radical procedure of allowing each of the evanescent proton-sized spaces thus obtained to be itself expanded to the size of our known universe, and to be granted a time-span of six billion years, within which it in turn became subdivided in both space and time just as we had previously subdivided our own known universe. The total number of genetic combinations that we could get in this way would now be the square of the previous number, and thus come to 4×10^{308}. But we should have to go on in this way, expanding protons into worlds and millimicroseconds into eons and then subdividing them as before, through about 14 cycles, before we attained the more conservative number, $10^{2,000,000}$, that we are seeking.

This result, then, may give us some glimmer of an image of how improbable we are. How right, then, in a short-sighted way, were those ostensible "savants" who, so they declared, found it "philosophically unsatisfying" to believe that they, or any other living things, had come about by accident. For what an unthinkable multitude of universes would have had to be searched through, before so improbable a combination of accidents as themselves could have been found. And yet, the near-magic faculty of multiplication by self-copying, possessed by the nucleotide chains, does give the opportunity for these most select combinations of accidents to arise. For the multiplication rate in *their* lines of descent *was* enough, had it been extended to all lines, to have produced that superlative number, of which our own combination formed just one unit. And after all, the persistence of the defective lines was not necessary for the outcome. In practical fact, on the contrary, their elimination was necessary.

5. **The role of sexual reproduction.** We may next inquire whether the period during which life has existed on the earth has been long enough to allow such a succession of multiplications as here required, that is, a one-hundred-fold multiplication occurring one million times in succession. Dividing these million steps among the three billion years or so during which fossil evidence indicates life to have existed

on the earth, we find 3000 years allowed, on the average, for each of the 100-fold multiplications. Now the number of generations occurring in every period of 3000 years has diminished from, potentially, millions, in stages corresponding to bacteria, to about 3000 (or 1 per year) for many of the lowlier many-celled forms, and then down to some 100 (or 1 in 30 years) in the case of modern man. At the same time, among many-celled forms, the potential amount of increase per generation has also diminished greatly. However, even modern man in America is now doubling his numbers every forty years, a rate which if continued would give a 100-fold increase in a mere 266 years. Of course, an advantageous mutant could seldom be expected to multiply so rapidly as this, relatively to the rest of the population. If, as seems reasonable, it had only a 1% reproductive advantage over the other individuals, it would require some 70 generations to achieve a doubling, and 465 generations for a 100-fold increase. This in man would occupy some 14,000 years. But since the human generation is so much longer than that which obtained in our ancestry until relatively recently, there was undoubtedly plenty of time for a million steps altogether.

We do become pinched for time, however, if we attempt, by this method, to squeeze in as many or more successful steps as our number of genetic nucleotide pairs, that is, some 4,000,000,000. For this would give only about a year, on the average, for each hundred-fold multiplication. If, as seems likely, each nucleotide pair has a history of several independent substitutions, and if by reason of the rarity of advantageous steps each period of multiplication requires an increase of 1000- to 100,000-fold rather than one of only 100-fold, then, as can readily be reckoned, each successful mutation would have had to double its numbers, on the average, every few days! This means that it would have undergone something like a 1% relative increase every hour.

Fortunately, the genes have found a way of meeting this evolutionary difficulty. Their answer is sex! Or rather, more precisely stated, it is sexual reproduction. The function of this arrangement is to expedite evolution by making it possible to obtain an accumulation of advantageous mutational steps without having the respective multiplications of these steps occur in series. They are allowed, instead, to occur in parallel, with concomitant interpenetration and combination of the respective lines of descent [8].

Let us first be clear concerning the basic genetic process involved in sexual reproduction. The act of fertilization that produces the child brings together two groups of chromosomes, or chains or genes,

of somewhat different ancestry. Although each of the two groups by itself comprises one virtually complete set of genes, there are some mutant genes present in each set. Now the mutant genes of one set are represented in the other set by a gene of the original type, or, more rarely, by a different mutant gene, lying at a corresponding position in a chain of that other set. At some time before the act of fertilization that results in the next generation—we shall call these the grandchildren,—the two sets of gene-chains line up parallel with one another, with their corresponding genes in apposition, a process called synapsis. In some viruses, at any rate, even the corresponding nucleotide-pairs lie in apposition at this stage. Following this synapsis, the apposed chains again separate, and they become distributed to different germ cells, each cell now receiving just one complete set instead of both of the sets that had come together.

The function of the coming together or synapsis is, for one thing, to accomplish an orderly separation, that insures each germ cell's receiving one complete set. But there is an even more important function in the synapsis. For, during their apposition, a considerable interchange of corresponding parts takes place among and between the chains of the two sets. Not only may a given germ cell thereby receive a whole chromosome (call it number 1), that had belonged to one set, and simultaneously another whole chromosome (2) of the other set, but many of the individual chromosomes that it receives are themselves mosaic. They are mosaic in the sense that their gene chain up to a certain gene, or nucleotide pair, has been derived from a given chromosome (call it number 3) of one of the sets, and from that point on has been derived from the corresponding chromosome (3) of the other set (see ref. [1]).

In higher organisms this interchange of chromosome parts, called crossing over, occurs by means of an actual breakage of the two corresponding gene-chains or chromosomes, one from each set, at the same point along their length, followed by the attachment of the left-hand part of one chain to the right-hand part of the other, and conversely between the other two parts. However, in the virus studied by Levinthal [5], the interchange is accomplished by the reproduction of the gene chains in such a way that one daughter chain is the daughter of one original chain up to a given point and of the other original chain from that point on. But in either case the outcome is the same. That is, each germ cell comes to contain, and bequeaths to the grandchild, just one complete set of genes, of which however certain ones trace back to the grandfather and the others to the grandmother on that germ cell's side. A grandchild, then, may receive

mutant genes from both sources at once, and it is able to transmit them together to its descendants. Remote descendants may thereby come to inherit this combination from both their parents, and to "breed true" for it, as we say.

Let us try to visualize mentally how this process expedites the accumulation of successful mutations. Suppose a horizontal row of dots at the top of a diagram represents a population at a given time, comprising n individuals, say 100,000. On the next line down are their descendants, averaging 1 apiece and therefore also n. If one *favorable* (i.e. advantageous) mutation occurs among f individuals, say 10,000, the number of favorable mutations in the first generation is n/f or 10. Suppose the reproductive advantage, r, averages 1%, in that 100 favorable mutants of this kind would in this setting tend to produce 101 offspring, as compared with 100 offspring from 100 non-mutants that did not have this competition. With this linear logarithmic increase it will take about 70 generations, on the average, before the number of these favorable mutants that arose in the first generation, 10, had been doubled to make 20. It is true, however, that the number of generations actually taken by the doubling would have a relatively high error. Moreover, many of the mutants would die out accidentally along the way while, as if to make up for these, there would be a much higher than average multiplication of some of the others. Here, however, we need consider only the averages. We may then ask the question: how long would it be before two favorable mutations had been accumulated in the same individual?

We shall take first the simpler case, that of organisms that do not reproduce sexually. In a case of this kind a second favorable mutation may be expected to arise in the same line of descent as that already containing one favorable mutation at such a time, on the average, as f individuals had been produced altogether, in that "line." That is, we do not have to wait until there are f (or 10,000) individuals of that line in one given generation but only until their sum in all generations has become f. The number of generations, g, required to attain this sum, f, is readily obtained, since g in this case represents the number of terms in a factorial series beginning with 1, in which each term is $(1+r)$ times the preceding term, and in which the sum of the terms is f. (Here $g=[\log (1+rf)/\log (1+r)]-1$.) Where, as in our numerical example, $f=10{,}000$ and $r=.01$, g, the number of generations required to accumulate 1 favorable mutation in addition to the first one, turns out to be approximately 464. Moreover, the number of generations, g_m, required for the accumulation of any given number, m, of such additional mutations, is simply mg (e.g. in our

example 9280 generations would be required for 20 of them). Or, conversely, $m = g_m/g$.

We may now compare this result with that in a sexually intermixing population having otherwise the same characteristics. In this case, by the time the generation g (or 464) is reached that in the asexual population would on the average have been necessary before a second favorable mutation was superimposed on the first one, there would have been a total population of gn individuals produced, and among all these there would have been gn/f favorable mutations. Now if we are dealing with a long period, of the order of tens of thousands of generations, such as those usually involved in considerations of "macro-evolution," we can ignore the length of time needed for any two favorable mutations of independent origin to become recombined so as to be present together in the same individual, or germ cell. For, in a relatively small fraction of such a period, the great majority even of mutations with as low an advantage as $r = .001$ would have had time to spread over practically the whole population. In so doing, these different mutant genes would have undergone the recombinations necessary to bind them together, that is, to incorporate them into the same chromosome sets.

Accordingly, in the sexual populations, virtually all of the gn/f favorable mutations arising during each period g (that in the asexual population allows just one more favorable mutation to accumulate) will have the opportunity of being eventually accumulated within the same descendants. Thus in any extended period represented as a large multiple of g, such as mg, the individuals of the sexual line can accumulate some mgn/f favorable mutations while those of the asexual line accumulate only m of them. That is, over a long period the speed of evolution in the sexual lines will be gn/f times that in the asexual lines. Even when a more unfavorable combination of numerical values is assigned to these terms than would often occur in practice (as when g is taken as only 10^2, n as only 10^6 and f as 10^6) this ratio is considerable (in this case 100). It may be inferred then that, ordinarily, sexuality increases the speed of evolution by a factor of many thousands and in some cases even millions. This enormous acceleration explains how it has been possible for several or many billions of mutations to have been accumulated by natural selection in the course of 3 billion years.

There is one factor that tends to make the situation even worse than this for the asexual as compared with the sexual population. This lies in the fact that in the former the favorable lines usually enter into an increasingly restrictive competition with one another,

thus reducing each other's selective advantage, whereas in the latter the formation of combinations of them tends increasingly to substitute cooperation for competition.

When a considerable period is under consideration, comprising tens of thousands of generations, the effective population number, n, to be used in the above formula, is that of practically the entire area between the parts of which any intermixing occurs, rather than the average number present within the partly isolated local groups usually dealt with in population-genetic studies. For in the course of the long period in question sufficient migration usually takes place between these groups to allow locally multiplied genes that would have a favorable influence in the group as a whole to become spread throughout the area. Because of the prodigious size attained, for many species, by the population of the all-inclusive area, the speed of their evolution becomes, over a long period, enormously enhanced by sexual reproduction. It should therefore be no matter for surprise that, having once arisen in primitive organisms, this procedure should have been retained by the great majority of species.

The above outlined mode of action whereby sexual reproduction allows evolution to proceed more rapidly has sometimes been misunderstood. According to this misconception, one of the ways in which sexual reproduction aids evolution is by allowing combinations to become formed and tried out, the individual genes of which would not have been advantageous in the general population but which, taken together, constitute a favorable complex. Undoubtedly there are many cases of such genes and they do play a significant role in evolution. It is to be noted, however, that in these cases there would be no greater opportunity for the lucky combination to arise in a sexual than in an asexual population. Only mutant genes that are advantageous in at least a local population, and thereby spread within it, have a greater chance of forming combinations through crossing than through successive mutations that occur in series as in asexual organisms. The fact that sexual reproduction is so widespread therefore attests to the great evolutionary importance of genes whose favorable effect does *not* depend on their presence in combination with other special mutant genes of independent origin. In other words, it attests to the prevalence of so-called additive effects of genes as opposed to complementary ones.

Sexuality got a far earlier start in evolution than was realized until, some thirteen years ago, the recombination process was discovered in bacterial viruses by Delbrück and soon afterwards in bacteria by Lederberg. Moreover, at about the same time, the idea arose [2c] and was later shown to be correct, that the so-called transforma-

tion of one line of bacteria by application of nucleotide chains from another is really a modified instance of the sexual recombination process.

It is true that some groups of organisms, including even higher organisms, in every period of the earth's history, have dispensed with sexual reproduction in fact or in effect, and that this has given them the considerable temporary advantage of being able to multiply without having to wait for the nuisance of finding and pairing with one another first. But these can have only a transitory splurge and are doomed to fall behind in the long evolutionary race and to disappear. They furnish an illustration of the shortsightedness, the opportunism, of natural selection. The stem forms of evolution, from which the organisms of later periods will be derived, are those that pay their tax to sexuality and are repaid in novel developments.

That even forms which have not undergone outwardly appreciable evolution for scores or hundreds of millions of years, such as some molluscs, have for the most part retained sexual reproduction, testifies to the continuing value for them of evolutionary adjustment of less tangible kinds. Among such adjustments are to be classed relatively temporary ecological adaptations, often largely invisible, that bring them into line with shifting conditions of their physical, chemical and biological environments. Sexual reproduction allows much prompter genetic accommodation of this kind. Another important group of changes in seemingly unchanging species are those, further discussed in §7, that result in improved regulatory responses, including both more accurate, wider range, and more versatile stabilizing mechanisms, and in the improvement of means of exploiting the environment. Such evolution is to a considerable extent cryptic, i.e. beneath the surface open to our present means of observation, for there are undoubtedly far more reactions of this kind in any organism than those of which we are aware. Progress in such directions must often involve selectional steps that individually confer only a minute advantage. Thus the selective pressure, being of only third or fourth order magnitude, requires, even with the aid of sexual reproduction, a very prolonged period for the achievement of important results. Nevertheless, taken together, these results, which the asexual species would be far slower still in attaining, may eventually be of decisive significance in the competition for survival.

6. **The importance of localized evolutionary experiments.** A factor materially affecting the establishment of advantageous mutations is the degree of subdivision of the species into semi-isolated groups. This factor, which has been treated mathematically by Sewall Wright

in numerous publications, is of especial importance in the case of genes of the type referred to in the 4th paragraph preceding, i.e. those that have a net favorable effect only as special combinations and not on the average when acting in connection with the genetic constitution of the population in general. We have seen that in such cases sexual recombination, operating widely throughout a large population, does not facilitate the formation and spread of these combinations. Yet in the long run such combinations are often of great importance, and if established may act as turning points that allow evolution to proceed in a new direction. As Wright has clearly shown, populational subdivision can greatly facilitate the establishment of these combinations.

There are two ways in which such subdivision can have this effect. For one thing, the number, n_l, of individuals in the local group is often so small as to allow some mutant genes that by themselves, even in connection with the genetic constitution of that local group, have no favorable effect, to become relatively numerous, merely as a result of the large random fluctuations to which small numbers are subject, a process termed "drift" by Wright. In some of these cases two or more such mutant genes which would be favorable only, or mainly, when in combination with one another, will thereby accidentally get the opportunity of being present together. This could of course happen just as well in asexual reproduction also. Having now become, as it were, superposed, their favorable joint action will come into play, so as to promote their spread. Under the circumstances of sexual reproduction, they can then spread much more rapidly and surely in the small group than if they were subject to the greater dissipation from one another that a larger group would entail. (A large asexual population, however, is not subject to this limitation.) Finally, by gradually diffusing out from the small group into its neighbors, and sometimes by the gradual advance of the local group as a whole into ever larger territories by competition, groupwise, with its less well equipped neighbors, these combinations can then proceed to "take over" in the general population, n.

The other and probably more important process depends on the many different selectional conditions to which the different local populations are subject. These tend to make some genes favorable from the start in a given local population that would not be favorable by themselves in the population as a whole. Both the peculiarities of the local environment (including the biological environment consisting of other species) and also the peculiarities of the genetic content of the local population itself (that arise as a result both of drift and of this very process of local selection) constitute important factors

in the causation of these selectional differences. In consequence of them, some combinations of these locally advantageous genes can become "established" in the sub-population (this time with the aid of sexual reproduction), which *as combinations* though not as separate genes would have a selective advantage even in the larger population or the species as a whole. And again, just as when such combinations had arisen through drift, these locally numerous combinations can then proceed gradually to spread throughout the species.

In both these ways, then, the subdivision of the large population into groups that are locally or temporarily more or less isolated from one another in reproduction allows the carrying out of numerous small-scale evolutionary experiments that would not have been permitted in the freely interbreeding or so-called "panmictic" large population. On the whole not as many evolutionary possibilities, nor as radical ones, are available for the reproductively undivided group. It is this latter type of population that mankind is rapidly approaching today.

The method of local experiments is not, however, the only way by which evolutionary corners can be turned and new directions embarked upon. For even to the large group new pathways may be afforded by alterations in conditions of living. These will oftener result from changes in the biological environment (that provided by other species) than in the inanimate environment, because the biological environment is so much more complex, diversified, and itself subject to change, than is the inanimate environment. The new pathways can also be presented, even to large relatively undivided species, when through the acquisition of given favorable mutations, or combinations of mutations that had been favorable even individually, the species acquires one or more faculties, or passes some threshold in the development of one or more faculties, that allows it to exploit a new mode of living.

Following any such turning of a corner there is likely to be a period of much faster evolution than before. For the longer a species has been selected for its old ways of life the harder it is to find new mutations that adapt it to these ways still better. On the other hand, for life carried out in a new way or (what amounts to much the same thing) under new conditions, many mutations that would previously have afforded little or no advantage will now be found helpful.

In such cases it is also much more likely to happen than before that the population, in its different parts, will find different methods of adapting to these new ways, and will find the new ways themselves to open up in diverse directions. Thereupon there will be a tendency not merely to faster evolution but also to a splitting of the

species into different lines. At first these lines will be isolated from one another in their reproduction mainly by geographic boundaries, but later genetic barriers (including genetically based physiological barriers) will arise between them as well.

The problem of how species split, in genetic terms, is one that is too ramified to permit of treatment here. It should be obvious, however, that the more numerous and the better isolated the local sub-populations are, the more such splitting is facilitated. It should also be observed that, the more any two sub-populations diverge genetically from one another, even in cases in which they retain great resemblances in their form and manner of functioning, the more likely they are to accumulate sets of genes that can no longer function effectively after having become mixed or recombined with one another [9]. We must recognize them as separate species after such mixing has become, in a state of nature, virtually impossible in consequence of these genetic incompatibilities.

7. **The development of stability and lability.** But the long course of evolution is by no means concerned only with the dramatic turning of corners and the multiple branching of pathways. Through the prolonged periods of seeming stasis there is, as noted in §5, a gradual genetic whittling away at structures and functions, an increasing refinement of them in adjustment to outer circumstances and to each other. Most of this is beneath the surface that is open to our present relatively crude means of inspection. Even at the turning of corners most of the individual mutations that succeed entail relatively small changes, since larger ones, despite being in some cases favorable in themselves, usually involve maladjustments of the already achieved delicate balances in the complicated interworkings of parts. Thus the other parts must gradually be changed correlatively, before a further change in the primary direction becomes profitable.

In consequence of these relationships the progression in a relatively new evolutionary direction is gradually enabled to go further and further. The appearance is thereby presented of an inner tendency to keep on varying genetically in the given direction rather than in other directions, a fiction denoted "orthogenesis," that has no sound basis in genetic reality.

It does remain true, however, that the organism because of peculiarities of its constitution is able to undergo genetic change much more rapidly in some directions than in others, and in some not at all. Moreover, this pattern of genetic inclinations is itself subject to change through mutation [10]. But these limitations are due largely to "canalizations" of its developmental and physiological processes,

if I may revert to my original meaning [7] of a term that has since then been used in diverse senses. Such inclinations, be it noted, can never force evolution to proceed in a given direction if the interests of the species are occasioning a selection that works in the opposite direction.

When evolution has not recently taken a radically new direction the visible alterations are, of course, still slower. Yet much is often going on beneath the surface, that may profoundly affect the embryological, the physiological, and the ecological reactions of the organism.

One of the most important classes of changes in this category concerns itself with the achievement of ever greater stability of development and of operation for structures and functions that are regularly needed. As pointed out long ago [11] and further emphasized since that time [12], mutant genes must gradually have been selected that gave greater stability and dependability both to the organism itself, in its characteristics, a property that might be denoted as "phenotypic stability," and also those that gave greater stability to the genes themselves, protecting them from the action of agents that might otherwise produce mutations in them.

Too much of a digression would be required for an adequate treatment of this matter in the present article. Suffice it to say that, so far as both kinds of stability are concerned, there is ground for inferring that many different mechanisms have been adopted: in fact, whatever came to hand. Some of these have had the effect of making the reactions in question especially resistant to disturbing influences. Others have worked by counteracting those influences themselves, somewhere along the line. Still others have involved self-regulatory or what are now termed "cybernetic" processes. Moreover, there has been, in phenotypic regulation, the attainment of "factors of safety," or means of doing the same thing through reactions that are somewhat different, involving more or less alternative pathways that are opened up where needed. The term "homeostasis" has often been applied to all this stabilization. It should be borne in mind that this term, like "adaptation" and "physiology" (all of which overlap widely) covers a fantastically great multitude of interwoven biological mechanisms. This complex is so impressive that it has sometimes been confused with the basis of life processes, of which it really forms a superstructure.[4]

[4] In its application to matters of genetic variation, the term homeostasis has recently been given a special meaning [13], whereby it denotes an essentially mystical doctrine, representing a revival from pre-Mendelian times. According to this doctrine, an organism's vigor is *per se* enhanced as a result of the hereditary elements derived from its two parents being unlike one another.

It must not be forgotten that stability is itself only a means of helping to insure survival and reproduction, and that oftentimes these ends are better achieved by lability. In fact, the entire set of physiological reactions of the organism serves as a grand series of examples of what may be called phenotypic lability [11a]. In each normal case, these reactions or changes that the organism undergoes in given situations, instead of representing the passive yielding to environmental pressures that is characteristic of inanimate objects, constitute adaptive responses. By this is meant responses that serve to fend off a danger to survival and/or multiplication, or that serve to take advantage of an opportunity to promote these end-results.

One might beg the question here by declaring that in these cases a deeper or higher stability is served, that of the species or genes themselves. However, this maneuver would stretch the term stability too widely inasmuch as these adaptations are ultimately directed not toward stasis but toward multiplication, of a kind that characteristically takes the form of expansion combined with evolution. It should also be emphasized that these adaptive changes, just like the stabilizing reactions (when, as is only sometimes the case, a distinction can be made!) represent no fundamental property of adaptation on the part of living matter. They are secondary developments, representing (as we have noted in the foregoing discussion) the consequences of the interminably repeated survival and multiplication of the mutant types that happened to be successful.

In addition to the adaptive reactions that have been developed with the function of promoting the welfare of the body proper there are of course those that promote its multiplication. And among the latter are some, of which the prime example is genetic recombination, implemented by sexual reproduction, the function of which is the facilitation of further evolution. Nevertheless, there are no grounds for suspecting that mechanisms have ever become developed that can direct the course of mutation into helpful rather than harmful channels. The process of mutation represents for the organism the taking of an untried step, to the consequences of which it is blind, but which it is ready to profit by if it should make a lucky strike.

It may be reiterated here that the favorable mutation is seldom a large one, and that the smaller its effect is, the more chance there is of its being helpful, and taking part in evolution [10b]. Never do highly organized structures that function helpfully in new ways come into existence at a bound, as they do in "science fiction" stories in which a child is born with telepathic antennae. All organs, tissues

and useful bodily reactions represent the remains of interminable trials, big and little errors that passed away, and little but accumulated successes.

8. **Mutation as destroyer or creator.** Because detrimental mutations are necessarily so much more abundant than favorable ones, it is evident that whenever the rate of elimination of the harmful mutant genes is slackened, as under conditions of easy living or artificial aids, their frequency will tend to rise, and the population will thereby fall off in its natural vigor and in the effectiveness of all reactions of types that have been thus protected [**14**]. For it is, in a sense, only selection that holds the body in shape, like the walls of a vessel containing a gas. Thus in the course of evolutionary time, "mutation pressure" will inevitably take advantage of any yielding of the selectional walls and allow the mass to lose its previous nicely adapted form, just as happens with the creatures who after countless generations in caves are found to be no longer capable, genetically, of forming functional eyes.

Sometimes these retrograde developments are all to the good, as when man, having adopted clothing, became relatively hairless, or when, following the practice of cooking and cutting his food, his great jaws receded. However, this process can be carried too far if society not only does all it can to help its genetically unfortunate members, as it certainly should, but if it also gives them every encouragement and assistance in passing along their weaknesses. By an indefinite continuation of this process, society would become overburdened. Moreover, it is evident that an increase in the pressure of mutations, caused for example by excessive radiation, would exert an influence that worked in the same direction as a relaxation of selection.

I have no fear that the course of mutational deterioration will go to serious extremes, because men are in the process of rapid learning. If they can now avoid self-made disaster they can enter a period of increasing hope and achievement. The rapid recent changes in their ways will cause them to revaluate their ancient standards. They will then see that, by realistically appraising both the world without and the world within themselves, by learning their own basic structure and reactions and the methods of controlling them, they can even challenge and improve upon the results of that greatest of creative operations, biological evolution itself. But first let them open their eyes and become aware of this living world for what it is.

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