CHAPTER 3

Mutations

Und was in schwankender Erscheinung schwebt,
Befestiget mit dauernden Gedanken.[1] GOETHE

'JUMP-LIKE' MUTATIONS – THE WORKING-GROUND OF NATURAL SELECTION

The general facts which we have just put forward in evidence of the durability claimed for the gene structure, are perhaps too familiar to us to be striking or to be regarded as convincing. Here, for once, the common saying that exceptions prove the rule is actually true. If there were no exceptions to the likeness between children and parents, we should have been deprived not only of all those beautiful experiments which have revealed to us the detailed mechanism of heredity, but also of that grand, million-fold experiment of Nature, which forges the species by natural selection and survival of the fittest.

Let me take this last important subject as the starting-point for presenting the relevant facts – again with an apology and a reminder that I am not a biologist:

We know definitely, today, that Darwin was mistaken in regarding the small, continuous, accidental variations, that are bound to occur even in the most homogeneous population, as the material on which natural selection works. For it has been proved that they are not inherited. The fact is important enough to be illustrated briefly. If you take a crop of

[1] And what in fluctuating appearance hovers,
Ye shall fix by lasting thoughts.

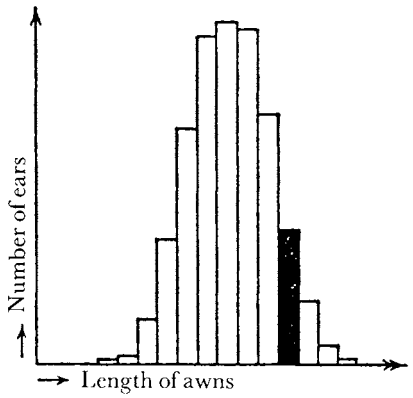

Fig. 7. Statistics of length of awns in a pure-bred crop. The black group is to be selected for sowing. (The details are not from an actual experiment, but are just set up for illustration.)

pure-strain barley, and measure, ear by ear, the length of its awns and plot the result of your statistics, you will get a bell-shaped curve as shown in Fig. 7, where the number of ears with a definite length of awn is plotted against the length. In other words: a definite medium length prevails, and deviations in either direction occur with certain frequencies. Now pick out a group of ears (as indicated by blackening) with awns noticeably beyond the average, but sufficient in number to be sown in a field by themselves and give a new crop. In making the same statistics for this, Darwin would have expected to find the corresponding curve shifted to the right. In other words, he would have expected to produce by selection an increase of the average length of the awns. That is not the case, if a truly pure-bred strain of barley has been used. The new statistical curve, obtained from the selected crop, is identical with the first one, and the same would be the case if ears with particularly short awns had been selected for seed. Selection has no effect – because the small, continuous variations are not inherited. They are obviously not based on the structure of the hereditary substance, they are accidental. But about forty years ago the Dutchman de Vries discovered

that in the offspring even of thoroughly pure-bred stocks, a very small number of individuals, say two or three in tens of thousands, turn up with small but 'jump-like' changes, the expression 'jump-like' not meaning that the change is so very considerable, but that there is a discontinuity inasmuch as there are no intermediate forms between the unchanged and the few changed. De Vries called that a mutation. The significant fact is the discontinuity. It reminds a physicist of quantum theory – no intermediate energies occurring between two neighbouring energy levels. He would be inclined to call de Vries's mutation theory, figuratively, the quantum theory of biology. We shall see later that this is much more than figurative. The mutations are actually due to quantum jumps in the gene molecule. But quantum theory was but two years old when de Vries first published his discovery, in 1902. Small wonder that it took another generation to discover the intimate connection!

THEY BREED TRUE, THAT IS, THEY ARE PERFECTLY INHERITED

Mutations are inherited as perfectly as the original, unchanged characters were. To give an example, in the first crop of barley considered above a few ears might turn up with awns considerably outside the range of variability shown in Fig. 7, say with no awns at all. They might represent a de Vries mutation and would then breed perfectly true, that is to say, all their descendants would be equally awnless.

Hence a mutation is definitely a change in the hereditary treasure and has to be accounted for by some change in the hereditary substance. Actually most of the important breeding experiments, which have revealed to us the mechanism of heredity, consisted in a careful analysis of the offspring obtained by crossing, according to a preconceived plan, mutated (or, in many cases, multiply mutated) with non-mutated or with differently mutated individuals. On the other hand, by virtue of their breeding true, mutations are a suitable material on which natural selection may work and produce

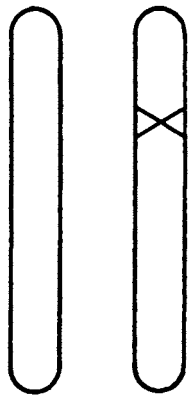

Fig. 8. Heterozygous mutant. The cross marks the mutated gene.

the species as described by Darwin, by eliminating the unfit and letting the fittest survive. In Darwin's theory, you just have to substitute 'mutations' for his 'slight accidental variations' (just as quantum theory substitutes 'quantum jump' for 'continuous transfer of energy'). In all other respects little change was necessary in Darwin's theory, that is, if I am correctly interpreting the view held by the majority of biologists.[1]

LOCALIZATION. RECESSIVITY AND DOMINANCE

We must now review some other fundamental facts and notions about mutations, again in a slightly dogmatic manner, without showing directly how they spring, one by one, from experimental evidence.

We should expect a definite observed mutation to be caused by a change in a definite region in one of the chromosomes.

[1]Ample discussion has been given to the question, whether natural selection be aided (if not superseded) by a marked inclination of mutations to take place in a useful or favourable direction. My personal view about this is of no moment; but it is necessary to state that the eventuality of 'directed mutations' has been disregarded in all the following. Moreover, I cannot enter here on the interplay of 'switch' genes and 'polygenes', however important it be for the actual mechanism of selection and evolution.

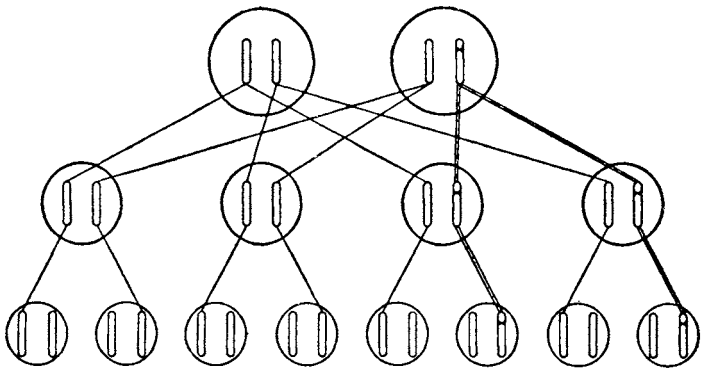

Fig. 9. Inheritance of a mutation. The straight lines across indicate the transfer of a chromosome, the double ones that of the mutated chromosome. The unaccounted-for chromosomes of the third generation come from the *mates* of the second generation, which are not included in the diagram. They are supposed to be non-relatives, free of the mutation.

And so it is. It is important to state that we know definitely that it is a change in one chromosome only, but not in the corresponding 'locus' of the homologous chromosome. Fig. 8 indicates this schematically, the cross denoting the mutated locus. The fact that only one chromosome is affected is revealed when the mutated individual (often called 'mutant') is crossed with a non-mutated one. For exactly half of the offspring exhibit the mutant character and half the normal one. That is what is to be expected as a consequence of the separation of the two chromosomes on meiosis in the mutant – as shown, very schematically, in Fig. 9. This is a 'pedigree', representing every individual (of three consecutive generations) simply by the pair of chromosomes in question. Please realize that if the mutant had both its chromosomes affected, all the children would receive the same (mixed) inheritance, different from that of either parent.

But experimenting in this domain is not as simple as would appear from what has just been said. It is complicated by the second important fact, viz. that mutations are very often latent. What does that mean?

In the mutant the two 'copies of the code-script' are no

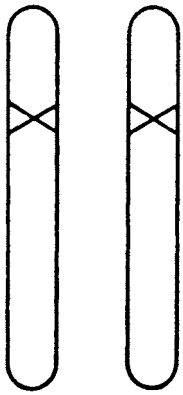

Fig. 10. Homozygous mutant, obtained in one-quarter of the descendants either from self-fertilization of a heterozygous mutant (see Fig. 8) or from crossing two of them.

longer identical; they present two different 'readings' or 'versions', at any rate in that one place. Perhaps it is well to point out at once that, while it might be tempting, it would nevertheless be entirely wrong to regard the original version as 'orthodox', and the mutant version as 'heretic'. We have to regard them, in principle, as being of equal right – for the normal characters have also arisen from mutations.

What actually happens is that the 'pattern' of the individual, as a general rule, follows either the one or the other version, which may be the normal or the mutant one. The version which is followed is called dominant, the other recessive; in other words, the mutation is called dominant or recessive, according to whether it is immediately effective in changing the pattern or not.

Recessive mutations are even more frequent than dominant ones and are very important, though at first they do not show up at all. To affect the pattern, they have to be present in both chromosomes (see Fig. 10). Such individuals can be produced when two equal recessive mutants happen to be crossed with each other or when a mutant is crossed with itself; this is possible in hermaphroditic plants and even happens spontaneously. An easy reflection shows that in these cases about

one-quarter of the offspring will be of this type and thus visibly exhibit the mutated pattern.

INTRODUCING SOME TECHNICAL LANGUAGE

I think it will make for clarity to explain here a few technical terms. For what I called 'version of the code-script' – be it the original one or a mutant one – the term 'allele' has been adopted. When the versions are different, as indicated in Fig. 8, the individual is called heterozygous, with respect to that locus. When they are equal, as in the non-mutated individual or in the case of Fig. 10, they are called homozygous. Thus a recessive allele influences the pattern only when homozygous, whereas a dominant allele produces the same pattern, whether homozygous or only heterozygous.

Colour is very often dominant over lack of colour (or white). Thus, for example, a pea will flower white only when it has the 'recessive allele responsible for white' in both chromosomes in question, when it is 'homozygous for white'; it will then breed true, and all its descendants will be white. But one 'red allele' (the other being white; 'heterozygous') will make it flower red, and so will two red alleles ('homozygous'). The difference of the latter two cases will only show up in the offspring, when the heterozygous red will produce some white descendants, and the homozygous red will breed true.

The fact that two individuals may be exactly alike in their outward appearance, yet differ in their inheritance, is so important that an exact differentiation is desirable. The geneticist says they have the same phenotype, but different genotype. The contents of the preceding paragraphs could thus be summarized in the brief, but highly technical statement:

A recessive allele influences the phenotype only when the genotype is homozygous.

We shall use these technical expressions occasionally, but shall recall their meaning to the reader where necessary.

THE HARMFUL EFFECT OF CLOSE-BREEDING

Recessive mutations, as long as they are only heterozygous, are of course no working-ground for natural selection. If they are detrimental, as mutations very often are, they will nevertheless not be eliminated, because they are latent. Hence quite a host of unfavourable mutations may accumulate and do no immediate damage. But they are, of course, transmitted to half of the offspring, and that has an important application to man, cattle, poultry or any other species, the good physical qualities of which are of immediate concern to us. In Fig. 9 it is assumed that a male individual (say, for concreteness, myself) carries such a recessive detrimental mutation heterozygously, so that it does not show up. Assume that my wife is free of it. Then half of our children (second line) will also carry it – again heterozygously. If all of them are again mated with non-mutated partners (omitted from the diagram, to avoid confusion), a quarter of our grandchildren, on the average, will be affected in the same way.

No danger of the evil ever becoming manifest arises, unless equally affected individuals are crossed with each other, when, as an easy reflection shows, one-quarter of their children, being homozygous, would manifest the damage. Next to self-fertilization (only possible in hermaphrodite plants) the greatest danger would be a marriage between a son and a daughter of mine. Each of them standing an even chance of being latently affected or not, one-quarter of these incestuous unions would be dangerous inasmuch as one-quarter of its children would manifest the damage. The danger factor for an incestuously bred child is thus 1:16.

In the same way the danger factor works out to be 1:64 for the offspring of a union between two ('clean-bred') grandchildren of mine who are first cousins. These do not seem to be overwhelming odds, and actually the second case is usually tolerated. But do not forget that we have analysed the consequences of only one possible latent injury in one partner of the ancestral couple ('me and my wife'). Actually both of

them are quite likely to harbour more than one latent deficiency of this kind. If you know that you yourself harbour a definite one, you have to reckon with 1 out of 8 of your first cousins sharing it! Experiments with plants and animals seem to indicate that in addition to comparatively rare deficiencies of a serious kind, there seem to be a host of minor ones whose chances combine to deteriorate the offspring of close-breeding as a whole. Since we are no longer inclined to eliminate failures in the harsh way the Lacedemonians used to adopt in the Taygetos mountain, we have to take a particularly serious view about these things in the case of man, where natural selection of the fittest is largely retrenched, nay, turned to the contrary. The anti-selective effect of the modern mass slaughter of the healthy youth of all nations is hardly outweighed by the consideration that in more primitive conditions war may have had a positive value in letting the fittest tribe survive.

GENERAL AND HISTORICAL REMARKS

The fact that the recessive allele, when heterozygous, is completely overpowered by the dominant and produces no visible effect at all, is amazing. It ought at least to be mentioned that there are exceptions to this behaviour. When homozygous white snapdragon is crossed with, equally homozygous, crimson snapdragon, all the immediate descendants are intermediate in colour, i.e. they are pink (not crimson, as might be expected). A much more important case of two alleles exhibiting their influence simultaneously occurs in blood-groups – but we cannot enter into that here. I should not be astonished if at long last recessivity should turn out to be capable of degrees and to depend on the sensitivity of the tests we apply to examine the 'phenotype'.

This is perhaps the place for a word on the early history of genetics. The backbone of the theory, the law of inheritance, to successive generations, of properties in which the parents differ, and more especially the important distinction recessive-dominant, are due to the now world-famous Augustinian Abbot Gregor Mendel (1822–84). Mendel knew nothing

about mutations and chromosomes. In his cloister gardens in Brünn (Brno) he made experiments on the garden pea, of which he reared different varieties, crossing them and watching their offspring in the 1st, 2nd, 3rd, . . ., generation. You might say, he experimented with mutants which he found ready-made in nature. The results he published as early as 1866 in the Proceedings of the *Naturforschender Verein in Brünn.* Nobody seems to have been particularly interested in the abbot's hobby, and nobody, certainly, had the faintest idea that his discovery would in the twentieth century become the lodestar of an entirely new branch of science, easily the most interesting of our days. His paper was forgotten and was only rediscovered in 1900, simultaneously and independently, by Correns (Berlin), de Vries (Amsterdam) and Tschermak (Vienna).

THE NECESSITY OF MUTATION BEING A RARE EVENT

So far we have tended to fix our attention on harmful mutations, which may be the more numerous; but it must be definitely stated that we do encounter advantageous mutations as well. If a spontaneous mutation is a small step in the development of the species, we get the impression that some change is 'tried out' in rather a haphazard fashion at the risk of its being injurious, in which case it is automatically eliminated. This brings out one very important point. In order to be suitable material for the work of natural selection, mutations must be rare events, as they actually are. If they were so frequent that there was a considerable chance of, say, a dozen of different mutations occurring in the same individual, the injurious ones would, as a rule, predominate over the advantageous ones and the species, instead of being improved by selection, would remain unimproved, or would perish. The comparative conservatism which results from the high degree of permanence of the genes is essential. An analogy might be sought in the working of a large manufacturing plant in a factory. For developing better methods, innovations, even if as

yet unproved, must be tried out. But in order to ascertain whether the innovations improve or decrease the output, it is essential that they should be introduced one at a time, while all the other parts of the mechanism are kept constant.

MUTATIONS INDUCED BY X-RAYS

We now have to review a most ingenious series of genetical research work, which will prove to be the most relevant feature of our analysis.

The percentage of mutations in the offspring, the so-called mutation rate, can be increased to a high multiple of the small natural mutation rate by irradiating the parents with X-rays or γ-rays. The mutations produced in this way differ in no way (except by being more numerous) from those occurring spontaneously, and one has the impression that every 'natural' mutation can also be induced by X-rays. In *Drosophila* many special mutations recur spontaneously again and again in the vast cultures; they have been located in the chromosome, as described on pp. 26–9, and have been given special names. There have been found even what are called 'multiple alleles', that is to say, two or more different 'versions' and 'readings' – in addition to the normal, non-mutated one – of the same place in the chromosome code; that means not only two, but three or more alternatives in that particular 'locus', any two of which are to each other in the relation 'dominant–recessive' when they occur simultaneously in their corresponding loci of the two homologous chromosomes.

The experiments on X-ray-produced mutations give the impression that every particular 'transition', say from the normal individual to a particular mutant, or conversely, has its individual 'X-ray coefficient', indicating the percentage of the offspring which turns out to have mutated in that particular way, when a unit dosage of X-ray has been applied to the parents, before the offspring was engendered.

FIRST LAW. MUTATION IS A SINGLE EVENT

Furthermore, the laws governing the induced mutation rate are extremely simple and extremely illuminating. I follow here the report of N. W. Timoféëff, in *Biological Reviews*, vol. IX, 1934. To a considerable extent it refers to that author's own beautiful work. The first law is

(1) *The increase is exactly proportional to the dosage of rays, so that one can actually speak [as I did] of a coefficient of increase.*

We are so used to simple proportionality that we are liable to underrate the far-reaching consequences of this simple law. To grasp them, we may remember that the price of a commodity, for example, is not always proportional to its amount. In ordinary times a shopkeeper may be so much impressed by your having bought six oranges from him, that, on your deciding to take after all a whole dozen, he may give it to you for less than double the price of the six. In times of scarcity the opposite may happen. In the present case, we conclude that the first half-dosage of radiation, while causing, say, one out of a thousand descendants to mutate, has not influenced the rest at all, either in the way of predisposing them for, or of immunizing them against, mutation. For otherwise the second half-dosage would not cause again just one out of a thousand to mutate. Mutation is thus not an accumulated effect, brought about by consecutive small portions of radiation reinforcing each other. It must consist in some single event occurring in one chromosome during irradiation. What kind of event?

SECOND LAW. LOCALIZATION OF THE EVENT

This is answered by the second law, viz.

(2) *If you vary the quality of the rays (wave-length) within wide limits, from soft X-rays to fairly hard γ-rays, the coefficient remains constant, provided you give the same dosage in so-called r-units*, that is to say, provided you measure the dosage by the total amount of ions produced per unit volume in a suitably chosen

standard substance during the time and at the place where the parents are exposed to the rays.

As standard substance one chooses air not only for convenience, but also for the reason that organic tissues are composed of elements of the same atomic weight as air. A lower limit for the amount of ionizations or allied processes[1] (excitations) in the tissue is obtained simply by multiplying the number of ionizations in air by the ratio of the densities. It is thus fairly obvious, and is confirmed by a more critical investigation, that the single event, causing a mutation, is just an ionization (or similar process) occurring within some 'critical' volume of the germ cell. What is the size of this critical volume? It can be estimated from the observed mutation rate by a consideration of this kind: if a dosage of 50,000 ions per cm^3 produces a chance of only 1:1000 for any particular gamete (that finds itself in the irradiated district) to mutate in that particular way, we conclude that the critical volume, the 'target' which has to be 'hit' by an ionization for that mutation to occur, is only $\frac{1}{1000}$ of $\frac{1}{50000}$ of a cm^3, that is to say, one fifty-millionth of a cm^3. The numbers are not the right ones, but are used only by way of illustration. In the actual estimate we follow M. Delbrück, in a paper by Delbrück, N.W. Timoféëff and K.G. Zimmer,[2] which will also be the principal source of the theory to be expounded in the following two chapters. He arrives there at a size of only about ten average atomic distances cubed, containing thus only about 10^3 = a thousand atoms. The simplest interpretation of this result is that there is a fair chance of producing that mutation when an ionization (or excitation) occurs not more than about '10 atoms away' from some particular spot in the chromosome. We shall discuss this in more detail presently.

The Timoféëff report contains a practical hint which I cannot refrain from mentioning here, though it has, of course, no bearing on our present investigation. There are plenty of occasions in modern life when a human being has to be

[1] A lower limit, because these other processes escape the ionization measurement, but may be efficient in producing mutations.

[2] *Nachr. a. d. Biologie d. Ges. d. Wiss. Göttingen*, 1(1935), 189.

exposed to X-rays. The direct dangers involved, as burns, X-ray cancer, sterilization, are well known, and protection by lead screens, lead-loaded aprons, etc., is provided, especially for nurses and doctors who have to handle the rays regularly. The point is, that even when these imminent dangers to the individual are successfully warded off, there appears to be the indirect danger of small detrimental mutations being produced in the germ cells – mutations of the kind envisaged when we spoke of the unfavourable results of close-breeding. To put it drastically, though perhaps a little naïvely, the injuriousness of a marriage between first cousins might very well be increased by the fact that their grandmother had served for a long period as an X-ray nurse. It is not a point that need worry any individual personally. But any possibility of gradually infecting the human race with unwanted latent mutations ought to be a matter of concern to the community.