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Origin of Genetic Variation: Regulation of Genetic Recombination in the Higher Organisms – a Theory

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Summary. Recent studies in the fungi, particularly *Neurospora* and *Schizophyllum*, have revealed a number of genetic features which, viewed in conjunction with earlier observations on other organisms, form a pattern, or model, which appears to be basic to the control of recombination in all eukaryotes, including higher organisms. It is assumed that the control is exercised on mechanisms that produce new alleles through recombination, as understood in broad terms and including such a likely phenomenon as gene conversion, which may or may not involve crossing-over, as well as equal and unequal crossing-over. The recombination may thus occur between alleles in either the homozygous or heterozygous condition. In the model, regulatory genes and breeding behaviour are integrated into one self-regulatory system controlling the production of new genetic variation.

The model is based on the following five general features, largely substantiated by the results in *Neurospora* and *Schizophyllum*: 1) The frequency of recombination in a particular chromosomal region is controlled by specific regulatory genes (*rec*). 2) There may be a number of such specific, regulatory genes responsible for recombination in a given region. 3) A *rec* locus may influence recombination in more than one region. 4) The regulatory genes have no specific physical relationship with the region(s) they control, and are usually located at random in the genome. 5) Of the allelic forms of the regulatory genes it is always the dominant gene which suppresses recombination and the recessive gene which increases recombination. The *rec* system is epistatic to other genetic elements jointly involved in the overall control of recombination in a specific region. It is suggested that usually the control of recombination in a given region is exercised, cumulatively, by the balance of the dominant and recessive genes of the specific *rec* loci in the organism. Outbreeding, with the associated high heterozygosity of the regulatory *rec* loci, virtually "switches off" recombination, producing few new variations. Inbreeding produces homozygosity of these loci, resulting in certain individuals which will have a considerable number of their regulatory loci in the homozygous recessive condition and in which recombination will be "switched on", producing new variation at a high frequency. Inbreeding is thus an integrated, evolutionary system of considerable importance, and is not a degenerate "dead end", as many investigators have previously thought.

The model has another compensatory function in evolution. In major loci, or in an operon, where there are structural genes and closely linked operator genes, as exemplified by the *S* locus, there are indications that the present model is concerned with the regulation of both structural and operator genes. The consequences of the model in the two classes of genes, however, are in direct contrast to each other: High heterozygosity which is instrumental in switching "off" recombination, and which is therefore helpful in maintaining stability in the structural gene, is conducive to functional variation of the operator gene; and high homozygosity, which is instrumental in switching "on" recombination, and which is therefore helpful in producing variation in the structural gene, is conducive to the stability of the operator gene.

This model of the control of genetic variation in a specific chromosomal region is significant in development as well as in evolution, and throws light on a number of hitherto "intractable" problems peculiar to the higher organisms. For example, the model is helpful in explaining: 1) the origin of new self-incompatibility alleles in the flowering plants; 2) the impressive speciation in the waif flora (and fauna) of the oceanic islands; 3) the presence of high genetic variability in inbreeding species of plants; 4) environmentally-induced heritable variation in certain plants; and 5) the genetic mechanism of antibody diversity in animals.

1. Introduction

Discoveries in the genetic mechanisms of microorganisms in the last two decades have greatly accelerated our understanding of the evolution and ordering of life processes. It has been generally assumed that the basic processes of life are similar, if not identical, in all living organisms. Thus, the recent findings in viruses, bacteria and fungi of the processes which control variability and expression of the genetic material have provided a fresh outlook on many problems posed by the higher organisms, which themselves are often not, with current knowledge and techniques, suitable materials for their elucidation.

Because large scale variability is generally achieved in nature through genetic recombination, which is largely based on meiosis, controls of genetic recombination are basically different in meiotic and non-meiotic systems. In non-meiotic systems, such as bacteria and viruses, there is considerable indirect evidence to suggest that mutations, rather than recombination, serve as the principal source of new genetic variability, while in meiotic systems the reverse is true (Simchen and Stamberg, 1969). Thus,

within micro-organisms, the picture in prokaryotes — e. g., the bacteria and viruses, is different from eukaryotes — the fungi.

Generally, in eukaryotes, which are meiotic, the complexity of the organism is related to the genetic organization of linkage systems in various functional and physical entities, e. g., major genes, supergenes or gene-complexes, linked or unlinked chromosomal regions which act together to produce a phenotypic character, chromosomes, and genomes. The survival of the eukaryotic line of evolution is therefore linked with the evolution of certain basic mechanisms controlling genetic recombination at these levels. The genetic studies in fungi, often the only eukaryote amenable to finer genetic analysis, are therefore specially significant in the understanding of the organization and control of genetic material in the higher organisms.

Certain recent investigations, particularly those of D. G. Catcheside and associates in the fungus *Neurospora crassa* (Catcheside, Jessop and Smith, 1964; Catcheside, 1966; Catcheside and Austin, 1969; Jessop and Catcheside, 1965; Smith, 1966; Jha, 1967; D. E. A. Catcheside, 1970; Angel, Austin and Catcheside, 1970) and of Simchen, Stamberg and others in *Schizophyllum commune* (Simchen, 1967; Simchen and Connolly, 1968; Simchen and Stamberg, 1969a, b; Stamberg, 1968, 1969a, b), have yielded information which appears to be very significant in the determination of intra- and interlocus recombination. Viewed together, these observations indicate an integrated system of control which is highly sensitive to the breeding system of the organism (whether inbreeding or outbreeding), and appears to be basic to all eukaryotes, including the higher plants and animals as well as the fungi. Based on these observations, taken in conjunction with similar observations in other organisms (for review, see Simchen and Stamberg, 1969), a genetic model of the control of recombination is presented. The model throws much light on a number of hitherto "intractable" problems peculiar to the genetics of higher organisms.

2. The Model

I. Background

In the present discussion the term "recombination" is used broadly and includes, among other mechanisms, such a likely phenomenon as gene conversion, which may or may not involve crossing-over (Whitehouse, 1963; Holliday, 1964). Recombination between two identical alleles may give rise to new forms of alleles, for example through unequal crossing-over. Separation and isolation of components of a complex locus may occur by an intrachromosomal mechanism such as that described for spontaneous mutations at the A_1 locus in maize (Rhoades, 1941; McClintock, 1950, 1957, 1961; Laughnan, 1961).

It is assumed with Simchen and Stamberg (1969), who critically reviewed the data on the genetic control of recombination, that there are two general types of control, 'coarse' and 'fine', and that all genetic factors affecting recombination could be assigned to one of these. The coarse control system, as defined here, consists of a large number of single genes which individually may have large effects, but not necessarily, as assumed by Simchen and Stamberg, always of the all or none type. The genes of this system influence recombination in the whole genome and are not concerned with specific chromosomal regions. For example, the system includes the control of the processes of chromosome movement, which govern the extent of close synapsis at the molecular level affecting frequency, terminalization and localization of chiasmata (Lindsley, Sandler, Nicoletti and Trippa, 1968). The control may also include aspects of the proper recombination process itself, possibly the later phase, where region specificity is not involved. These genes together control the occurrence and sequential procession of general events that are essential to the process of recombination. They control activities that precede, follow, or are a part of, the recombination process but which are general and not region-specific. They are often revealed by the occurrence of rare mutants that depart severely from normal behaviour in recombination. The *rec*⁻ mutants of *E. coli* (Clark, 1967), the *c3G* mutant of *Drosophila melanogaster* (Gowan, 1933), and cytological asynaptic or desynaptic mutants in barley, tomato and other plants (Riley and Law, 1965), examplify this. These mutants are believed to be in the nature of a defect or absence of a certain substance or enzyme essential for the recombination process (*E. coli* and *D. melanogaster*), or a defect or loss of a step in the normal sequence of meiosis (asynapsis or desynapsis).

The fine control system affects the level of recombination in a more specialized way. Possibly it is concerned with the early phase of the recombination process proper, where region specificity is essential (D. G. Catcheside, Pers. Comm.). It is believed to be superimposed on the coarse control, which is considered to be primitive and more characteristic of prokaryotes. Since discriminatory recombination is one of the most important elements in the evolution and stability of complex organization, the fine control system is an integral part of the evolutionary process which has given rise to higher organisms.

The fine control system is best exemplified by the recent results in fungi. In *Neurospora* several *rec* (recombination) genes controlling allelic recombination frequencies have been identified. Some at least of these *rec* genes also influence intergenic recombination frequency. The present evidence from *Schizophyllum* and *Neurospora* strongly suggest that both intra- and intergenic recombination are influenced by the *rec*-type genes.

The *rec* genes are highly region-specific but the relationship is not one-to-one: that is, one *rec* gene can control recombination in several chromosomal segments. Thus the number of *rec* loci need not be very great.

The segments which are controlled by the same *rec* locus may be loosely linked to the latter, or may be located at random in the genome. Whitehouse (1966) has suggested that the controlled regions correspond to units of transcription, that is, to operons, but there is also evidence to the contrary (D. E. A. Catcheside, 1968). It is likely that the segments which are jointly controlled have some common role with regard to a particular fitness character (Simchen and Stamberg, 1969), and that their common control is a result of the natural selection involved in the co-ordinated evolution of the phenotypic features bestowing that fitness characteristic.

The increase in frequency of recombination varies between the allelic forms of different *rec* loci but it may be as high as 25 times or more. Thus the fine control allows frequencies of recombination in a particular region to vary within a wide range.

At present there is inconclusive evidence that there may be a number of loci controlling recombination frequency in the same region (D. G. Catcheside, Pers. Comm.). However, Simchen and Stamberg (1969) have concluded that in order "to explain the shapes of the distributions of recombination frequencies, it must be assumed that allelic differences at several loci are regularly used in the control of frequency of recombination for a given region." It is suggested here that the several loci presumed to be involved in the control of recombination in the same region may arise from two sources. 1) There may be different functional components of the fine control system each regulated by one or more separate loci. There are a number of known factors — e. g., distance from the centromere, crossing-over interference, heterochromatic regions, B-chromosomes and long-inversions (Rees, 1961; Sun and Rees, 1964; Riley and Law, 1965) — which affect the frequency of recombination in a particular region. 2) Some of the loci may have originated as duplicates of other original loci and later been scattered in the genome through translocation.

The natural variation found in the fine control, as shown by many observations in the eukaryotes, is one of its characteristic features. At least two alleles must exist concurrently in a population for every genetic difference (Simchen and Stamberg, 1969).

II. The Main Feature

A major distinction between the loci involved in the control of coarse and fine control systems, which is highly significant in the present model, is the characteristic and contrasting dominance relationship between the allelic genes in the two systems. In the coarse control system the dominant genes *increase* recombination frequency and the recessive genes *restrict* it; in the fine control system the dominant genes *restrict* recombination frequency and the recessive genes *increase* it (Fig. 1).

Wherever components of polygenic variation involving coarse control of recombination, especially chiasma frequency, have been studied, the dominance is in the direction of higher recombination, as demonstrated in *Secale* (Rees and Thompson, 1956; Rees, 1961; Sun and Rees, 1964), *Hordeum* (Gale and Rees, 1970), *Drosophila* (Law, 1961; Lawrence, 1963) and *Neurospora* (Stadler and Towe, 1962). In genetic variations which exclude recombination altogether, and which are due to a single major deleterious gene (examples cited earlier, *E. coli* — Clark, 1967; *D. melanogaster* — Gowan, 1933; flowering plants — Riley and Law, 1965), dominance is again in the direction of normal chiasma frequency while the recessive genes eliminate chiasma formation.

In the fine control system in *Neurospora*, "in every case, whether the effect is upon allelic or non allelic recombination, the dominant *rec* gene (*rec*$^+$) reduces recombination" (D. G. Catcheside, Pers. Comm.). In the *Schizophyllum* system of Simchen and associates, where only differences in interlocus recombination are studied, again there is evidence suggesting that low frequencies of recombination are dominant to high frequencies.

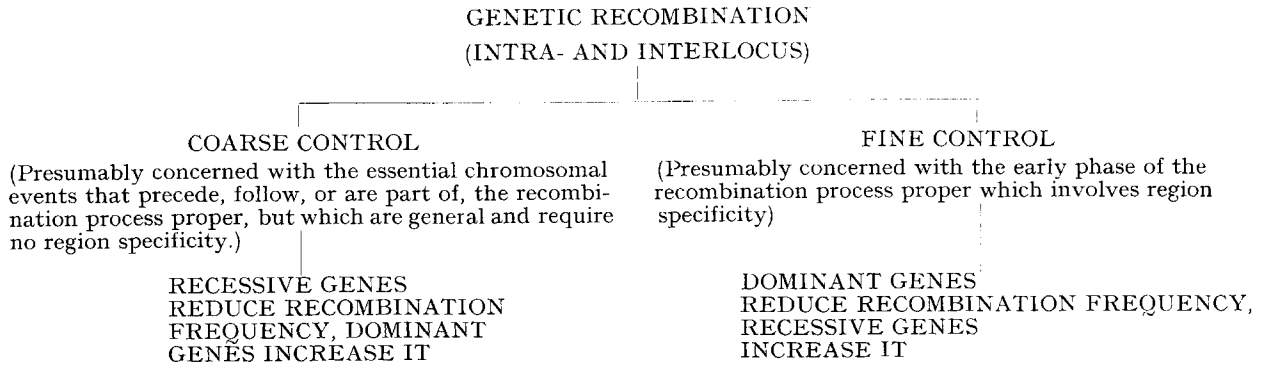


Fig. 1. A generalized picture of the control of genetic variability

Angel, Austin and Catcheside (1970) have recently reported the presence of another gene, *cog*, believed to be of the nature of recognition site, which is closely linked to the *his-3* locus and which, together with the *rec* gene, appears to be involved in the control of recombination at the *his-3* locus. There is a dominance relationship between the *cog* alleles, the dominant form cog^+, in contrast to rec^+, increasing recombination frequency. From the point of view of the present model, what is significant is the fact that the overall control is still through the dominance relationships of the *rec* genes, for rec^+ is epistatic to cog^+. When the rec^+ gene is absent the cog^+ may express, and may thus bring about some additional flexibility to the system.

Thus, there appears to be a basic difference in the *overall control mechanisms* of coarse and fine regulation of the recombination process: *in the fine regulation system the recessive genes increase recombination frequency, in the coarse regulation system they reduce it.* It is suggested here that this basic difference between the two systems is of considerable evolutionary significance.

Apart from a certain degree of regulation of recombination, there is possibly another aspect to the coarse control system. Because a single recessive gene of the coarse control system may bring about a serious breakdown of recombination, and cause sterility, it is very rarely that instant expression of such a gene could form an integral part of a viable evolutionary line. However, a singular exception is the well known situation in certain insects, where no recombination occurs in one of the sexes, for example, in males of *Drosophila melanogaster*. The presence of several loci, with the dominant form of the allelic genes imposing near-normalcy, provides for interlocus and interallelic interactions (e. g., dominance, epistasis, complementation, heterozygote advantage), possibly resulting in genetic mechanisms controlling the size of populations under different breeding and ecological conditions.

The reverse allelic relationship in the fine control system, where dominant genes restrict recombination and recessive genes increase it, has an apparent adaptive value, and appears to be of great evolutionary advantage. The present model is based essentially upon this characteristic of the fine control system.

The fine control system in the eukaryotes generally is considered to consist of regulatory genes of the *rec* type, as discussed above. It is assumed that there are usually several *rec* loci which, cumulatively, control the frequency of recombination in a given chromosomal region, including one or more structural and other forms of loci. Normally, in the outbred population the organism will be heterozygous for all or most of these *rec* loci, thereby producing the dominant rec^+ phenotype, which would result virtually in the "switching off" of recombination in the region. Inbreeding will produce homozygosity of the *rec* loci, resulting in certain individuals having considerable numbers of the *rec* loci in the homozygous recessive condition. In these individuals recombination will be "switched on", producing new allelic and non allelic variation at a high frequency. Recombination frequency may be entirely a function of the proportion of the homozygous recessive *rec* loci present, or there may be a threshold over which recombination is switched on and below which it is switched off.

Thus the evolution of specific dominance and epistatic relationships (Gregg, 1967) between the genes jointly involved in the control of recombination in a particular chromosomal region is of critical significance in the model.

Some of the implications, and specific adaptations, of the model in resolving certain problems in higher plant and animal genetics are considered below.

3. Self-Incompatibility

I. New Self-Incompatibility Alleles

One of the puzzles of higher plant genetics is the mechanism by which new self-incompatibility alleles of the *S*-gene complex arise. The *S*-gene complex is a closely linked, physiologically integrated unit, an operon, comprising structural gene(s) coding for specificity and at least two operator genes, one controlling the activity of the structural gene in the pollen, the other in the style. It controls breeding behaviour and is believed to have literally hundreds of alleles. Yet, extensive studies of spontaneous and *x*-ray induced mutations have not revealed one definite case of mutation from one self-incompatibility allele to another. Mutations found were all in the operator cistrons which control activity of the *S* complex, and which resulted in the breakdown of the system bringing about self-compatibility, but none in the structural cistron which controls the specificity of the *S* alleles (Lewis, 1954, 1960; Pandey, 1956, 1965, 1969a, 1970b; Brewbaker and Natarajan, 1960).

There have been reports suggesting that new *S* alleles arose in inbred *Trifolium pratense* (Denward, 1963), *Lycopersicum peruvianum* (de Nettancourt and Ecochard, 1969) and *Nicotiana bonariensis* (Pandey, 1970a). This indicated that inbreeding was involved in the origin of new *S* alleles. The problem was, how. The present model explains the genetic mechanism by which inbreeding could be instrumental in creating new alleles. A preliminary report in this connection has appeared before (Pandey, 1970a).

The proposed model not only provides a basis for the origin of new *S* alleles but also has the important evolutionary advantage of being self-regulatory. When the *S* alleles are already numerous, as in a relatively large interbreeding population, the high heterozygosity would suppress recombination in the structural cistron, thereby virtually shutting off the production of new *S* alleles. In an isolated small

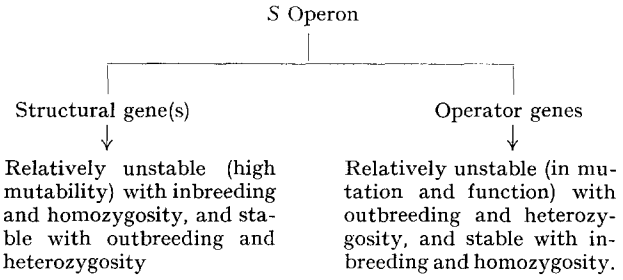
population having only a few *S* alleles, inbreeding would trigger a chain of events which would lead to the release of the structural cistron from the suppression of recombination, thereby generating new *S* alleles with a high frequency. Thus, a relatively small group of self-incompatible plants isolated from the larger community of the parent population can, in time, rebuild the supply of *S* allelic variation.

In certain extreme situations, for example, in the case of single plants or a few scattered individuals, pseudoself-compatibility resulting from interspecific hybridization, competitive interaction in diploid pollen, or from environmental, end-season, or other physiological causes, may give rise to inbred plants and thus eventually trigger the production of new *S* alleles. Even rare production of seed through selfing as brought about by revertible mutations, cytoplasmic effects, or other genetic causes would be advantageous (Pandey, 1959, 1968b, 1970b). Almost all self-incompatible species investigated so far have shown the ability to produce some seed when subjected to sustained self-pollination, a condition specially present in large clonal colonies of plants. In this connection it is clear that an association of self-incompatibility with perennial habit and vegetative reproduction would be of considerable advantage, and self-incompatible species with these characteristics would be favoured in dispersal and successful colonization of new habitats. *Under these conditions, the more widely scattered the distribution of a species the more alleles the species as a whole will have* — a generalization illustrated by *Trifolium repens* (Atwood, 1944; Lewis, 1954; Pandey, 1956). The model explains how *T. repens* could have its present hundreds of alleles, although presumably it started as an allotetraploid from crosses between two self-incompatible species and hence with possibly as low as two different *S* alleles. It is interesting to note that *T. repens* is a perennial, has the ability to reproduce vegetatively, and has a wide geographical distribution.

II. Regulation of the Operator Genes

The model also suggests that at least a part of the polygenic component of the incompatibility system (Mather, 1943, 1944, 1966) includes regulatory genes of various types, one of which may be the *rec* type gene controlling recombination in the structural cistron of the *S* operon. Crowe's work (1970) on *Borago officinalis* suggests that the expression and mutation of the operator genes of the *S* complex may be controlled by certain regulatory genes. *B. officinalis* is predominantly outbreeding and individuals show continuous variation in their degrees of self-compatibility. Inbreeding reveals a striking correlation between self-incompatibility and homozygosity on the one hand and self-compatibility and heterozygosity on the other. This may suggest that the operator genes are much more susceptible to alteration when the plants are polygenically heterozygous than when they are homozygous.

The two major classes of elements in the *S* operon may, therefore, behave differently in response to the breeding system and the consequent composition of the polygenic component, as shown below: —



The above conclusion is also supported by the *S* gene mutation studies in several species (Lewis, 1954, 1960; Pandey, 1956, 1965, 1967; Brewbaker and Natarajan, 1960). The highly heterozygous self-incompatible plants used in these studies produced only mutations of the operator genes resulting in self-compatibility. No mutation of the structural gene was recovered. This would suggest that there is independence of regulation within the operon component of the genetic system, between the two classes of elements, the structural gene and the operator genes.

The present model, based on the opposing consequences of heterozygosity and homozygosity of the population, is, therefore, concerned not only with the capability of generating a large amount of variability for a locus, expressed basically in the form of different but related proteins, the prerogative of the structural cistrons, but also with the alternatives of producing or not producing a protein, the prerogative of the operator cistrons. It thus covers both qualitative aspects of gene action and is concerned with a whole operon, or a larger segment, controlling an integrated unit of function.

4. Inbreeding

The great majority of wild organisms, plants and animals, are outbred. There are, however, especially among plants, many local trends towards restriction of recombination, leading to closer adaptation but lower adaptability. The ecological relation of such trends — for example, as a function of life cycle, breeding system and geographical distribution — has been discussed by many authors, notably Darlington (1958), Darlington and Mather (1952), Mather (1943, 1953, 1966), Stebbins (1950, 1958, 1960), Lewis and Crowe (1958), Grant (1958), Baker (1959a, b), Fryxell (1961) and Antonovics (1968). However, the success, frequency and, in many cases, antiquity of many groups of plants which are predominantly self-fertilized may not be entirely explained on the bases of the advantages hitherto discussed.

I. Waif Flora of Oceanic Islands

One of the problems not satisfactorily explained is the impressive extent of speciation on the waif flora of oceanic islands, for example, Hawaii and New Zealand (Davis, 1950; Darlington, 1965; Carlquist, 1966). As Carlquist (1965) states "Dispersal to an oceanic island is like a filter, narrowing down to a handful of species those which arrive and establish. Somehow this process must be reversed in order to achieve the diverse and novel biological productions living today on remote islands. The mechanism is, of course, evolution". Probably one of the most significant "filters" through which these plants are required to pass is self-compatibility, without which stray, single propagules are unlikely to start a sexually reproducing colony. The significance of this idea in long distance dispersal, first proposed by Baker (1955), is celebrated in what Stebbins (1957) has called "Baker's Law."

The paradox of waif flora is the extremely small amount of genetic variability that can be introduced with the stray establishment of single, or in rare cases a very few closely spaced, propagules of a species. These must eventually form a nucleus stock for future evolution, probably in some cases augmented by further such introductions in space and time. Self-compatibility and self-pollination, while extremely helpful in the initial establishment of a rare propagule after long distance dispersal, further limit the scope of genetic variability.

The present model suggests that enforced inbreeding of the initial waif flora of the remote oceanic islands created the genetic situation, which through a relatively high 'mutation' rate, promoted an increased production of genetic variation. This, to a considerable extent, offset the disadvantages inherent in the extremely small number of original immigrant plants. In time, variation similar to that which occurred in the original homeland may be repeated in the new adopted land, but since the genetic and ecological situation may be vastly different, selection is most likely to yield different end products resulting in speciation.

II. Genetic Diversity in Inbreeding Populations

The recent elegant experiments of Allard and associates (Allard, 1965, 1966; Allard, Jain and Workman, 1968) have shown that there is remarkable genetic diversity within natural and domestic populations of inbreeding species. Quantitative studies of inbreeding species revealed that any given population contains individuals of many different genotypes. Individuals within populations often differ from one another by single- and multiple-unit polymorphisms and also by continuously varying characters, and are frequently heterozygous at many loci. Thus the population structure in inbreeding species comprises co-adapted gene pools, evolution of which is much more complicated than has been commonly supposed (Allard, 1965). The demonstration that inbreeding species are genetically highly variable challenges a number of important theoretical and evolutionary considerations of the role of inbreeding, which hitherto were based on the assumption that inbreeding populations are genetically uniform.

Theoretical considerations, supported in several instances by practical observations, suggested that selection involving heterozygote-advantage may help to explain the existence of stable polymorphisms under intensive inbreeding, and that there may be interaction between different polymorphisms. Allard *et al.* (1968) concluded that maintenance of a stable non-trivial polymorphism depended on a complex set of interactions between genetic factors, mating system, and ecological factors including frequency dependent selection.

The discovery that remarkable genetic variability exists within populations of inbreeding species has led Allard (1965) to an important conclusion, that "high variability is essential to the survival of populations". The question arises: what is the primary source of the *origin* — apart from *maintenance* — of such variation in predominantly inbreeding populations? Allard and associates believe that rare hybridization is the source. However, there is a serious doubt, as shown from experiments on *Festuca microstachys* complex (Kannenberg and Allard, 1967), whether rare outcrossing alone can be considered to be sufficient to generate the high degree of variation observed in the populations of these species. In the *F. microstachys* complex, which is cleistogamous, it is considered that the rate of outcrossing is probably lower than one per 10,000 fertilizations. Yet, Kannenberg and Allard's (1967) comparative study of grasses indicated that these fescues were no less variable in comparable quantitative characters than either wild oats, a species in which the extent of outcrossing is many times higher (1—10%), or *Lolium multiflorum* (Schulke, 1963), an outcrossing species. The explanation that rare cross-pollination is the source of genetic variation, therefore, appears untenable. This problem has led Allard *et al.* (1968) to another significant conclusion, that "change of any component of the variability system which leads to undue restriction of variability must be mediated by compensatory changes in other components if the population is to survive". However, these authors believe that the component factors involved "are more ecological than genetic", implying that the answer to this dilemma may be found in ecological genetics rather than in any purely genetical phenomenon. The present model suggests that this may not be so.

Evolution needs variation which in turn depends on mutation and recombination. When the collective mutation potential of a species is denied in the creation of large-scale variability through recombination, as in a self-pollinating species, the extent to which

usual mutation rates can generate variability is correspondingly diminished. Interspecific or intra-complex hybridization as a means of acquiring genetic variation is also severely limited in an inbreeding population adapted to self-pollination. Is, then, the loss of variation in an inbreeding population offset in nature, at least partially, by some other purely genetic means not yet evident?

The present model suggests that the evolution of breeding behaviour is linked with the evolution of a self-regulatory genetic mechanism controlling recombination in major (structural) loci. With outbreeding and increase in heterozygosity the frequency of 'mutation' of the structural loci coding for protein specificity is drastically reduced. Conversely, the introduction of inbreeding and the resultant homozygosity greatly increases the 'mutation' rate of these loci. Thus, inbreeding populations would have a relatively much higher 'mutation' rate in their structural genes than outbreeding populations, perhaps reaching a climax in the cleistogamous species such as the *Festuca microstachys* complex. As pointed out earlier, the model postulates that inbreeding is conducive to the stability of the operator genes, which would further ensure that the essential basic physiology of the functionally important polymorphic genes would not be destroyed under strong selection pressure.

The model may have some significance in explaining the success of inbreeding weeds in colonizing unsuitable habitats, and also the success of apomixis as an alternative to self-fertilization for achieving a similar end. In apomicts, which are usually highly heterozygous, the model predicts that, contrary to the situation in inbreeding species referred to above, 'mutations' of the operator genes are likely to be of much higher frequency. Since apomicts are often polyploids, 'mutations' of the operator genes may be complemented by the duplicate genes in the genome thus providing a variety of functional variations (Pandey, 1969a, d). The 'mutational' variability could arise primarily through breakdown of the originally complex processes. Apomicts may thus have inherently gained some degree of compensation for the loss of their continuous sexual variability; and their widespread success (Clausen, 1954) may, at least in part, be attributable to their ability to generate variability at this level. The model, which combines genetic recombination and breeding behaviour into one integrated system, may have great significance in the evolution and speciation of plant kingdom as a whole.

In the essentially outbreeding animal kingdom the mechanism provided by the model would protect the large, outbreeding, heterozygous populations from increasing the already heavy load of deleterious genes, and thus contribute towards stability. In animals, only in extreme conditions of isolation of small populations, resulting in inbreeding and polygenic homozygosity, would it create variability, and hence promote adaptation and possible speciation. This may be particularly relevant in relation to speciation in the waif fauna of the distant oceanic islands.

5. Polygenes and Quantitative Characters

The original concept of Mather (1949) distinguishing between major genes and minor or polygenes has been amply substantiated by results in micro-organisms. Rendel (1968) reviewing this topic considers a major gene as one with large effects and which is regulated, and regards it as a structural gene in the sense used in bacterial genetics. Specific developmental processes are initiated by a particular major gene, other genes taking part in the process being, in one way or another, regulatory in function.

There may be at least three types of polygenes regulating a major gene, or an operon: 1) Specific genes which affect the qualitative expression of a major gene by controlling its intragenic recombination frequency (mutation), for example the *rec* type genes in the present model. These regulatory genes may have allelic differences determining their dominance relationships and their effects are cumulative. 2) Specific genes which affect the quantitative expression of a major gene by controlling the rate of protein synthesis. They may have similar allelic differences as in 1). 3) Non-specific genes which affect a major gene indirectly through interconnections of metabolic pathways.

Consideration of the present model in relation to the above types of regulatory polygenes suggests not only a mechanism for the origin of polymorphism, as in the case of the *S* locus, but also a mode of origin of "quantitative characters" from the originally "qualitative characters".

It may be assumed that initially each structural gene had at least two specific regulatory genes, one, for example *mut*, here denoted by *M*, controlling the qualitative variation (mutation) and the other, *qun* (*Q*), controlling the quantitative expression. If polymorphism of the protein coded by the structural gene had a selective advantage it could arise through point mutations, at the simplest level, by having two alleles *M* and *m* at the *mut* locus, where the dominant gene produces stability and the recessive gene produces a certain specific rate of mutation. Certain, relatively few, rates of mutation can be produced by generating a small number of alleles at the *mut* locus. This system will give rise to discontinuous mutation rates and, from the point of view of selection, will have a relatively low flexibility.

In cases where evolution of continuous mutation rates, having high flexibility, has a selective advantage, as in the case of the *S* locus — and which is probably true of most major loci — it could arise by the following chain of events: duplication of the *mut* locus, mutation of the duplicate gene, and dispersal of the new gene in the genome through transloca-

tion (the last two events not necessarily in the same order). In this way a relatively limited number of *mut* loci, with varying effects and dominance, may provide a pool of genes in the genome from which different combinations, cumulatively causing varying rates of mutation, can be selected. This is the evolutionary course of events envisaged for the control of mutation rate of the structural cistron of the *S* complex and other similar highly polymorphic structural genes (the latter presumably themselves being relatively long DNA chains having arisen from an initially smaller chain through the process of duplication and differentiation). These regulatory *mut* genes would be comparable to *rec* genes of the present model. If variability in the quantitative expression of a structural gene has a selective value it can arise through a similar chain of events as above, at the *qun* locus.

Under favourable selection pressures, transformations of a qualitative into a quantitative character, and of a monomorphic into a polymorphic character, may thus be achieved through evolution of the initial regulatory genes into polygenes. Duplication of genes and their subsequent mutation, and often dispersal in the genome, is an essential process in the development of genetic systems, and in the evolution of complex organisms generally (Ohno, Wolf and Atkin, 1968; Stebbins, 1968; Pandey, 1968a, 1969a, b, c, d).

Identifying the regulatory genetic systems in crop plants, and a better understanding of them, raise the possibility of isolating strains through which a great deal of variability in a particular qualitative character can be obtained, *e.g.*, a useful enzyme, amino-acid, colour or any other product determined by a structural gene. Mutation in regulatory genes may result in higher rates and duration of synthesis for particular proteins, for example that of photosynthetic processes. It also raises the possibility of new combinations of cumulative genes which may give rise to extraordinary phenotypes reflected in quantitative characters such as yield, grain size, and other characters of agronomic value. Thus manipulation of controlling elements and regulatory systems, if they can be placed under experimental control, could result in increased yields of food and forage plants on a scale hitherto unimaginable with the present techniques.

6. Mechanism of Antibody Diversity

One of the most fascinating problems in mammalian genetics is the nature of the genetic control of antibody variability. Vertebrate organisms appear to be capable of synthesizing thousands of different antibody sequences, each presumably determined by a different antibody gene. The problem is how do these genes arise. In the main there are two theories:

(1) The germ line theory postulates that vertebrates have a separate germ-line gene for each antibody polypeptide chain the animal is capable of elaborating. Hood and Talmage (1970) have discussed the possibility of the presence of 20,000 genes, comprising approximately half light chains and half heavy chains, which through unrestricted combination could generate as many as 10^8 ($10^4 \times 10^4$) different antibody molecules. They argued that this was not an excessive demand on the total DNA of the germ cell, being only 0.2 per cent of the genetic material of human haploid cells.

(2) In contrast, the somatic theory of antibody diversity postulates that antibody genes arise by hypermutation from a relatively few germ line genes during somatic differentiation (Burnet, 1969).

Both theories assume that the extremely high number of antibodies produced are the ultimate products of combinations between fewer units, which themselves, from the point of view of evolution, possibly originated initially from one common molecule encoded by a primitive cistron (Burnet, 1969, 1970; Gally and Edelman, 1970). If there is a threshold so that the extent of the genetic material required by the germ line theory cannot be carried by a genome, as is likely, the somatic theory would appear to be the answer. The main problem with the somatic theory, then, lies in the mechanism explaining hypermutation (Burnet, 1969).

It is suggested here that the genetic requirement of somatic hypermutation can be met on the basis of a specific adaptation of the present basic model early in the evolution of vertebrates. In line with the discussion in the previous section, it is assumed that there are only two alternative alleles of a small number of regulatory genes controlling the rate of recombination in the presumed specific structural cistronic complexes involved in the determination of antibody sequences, the dominant allele *M* which suppresses recombination and the recessive allele *m* which produces an extremely high rate of recombination. In vertebrate animals, which are as a whole unisexual, all individuals would be invariably heterozygous for one or more of the regulatory genes.

It is suggested that the presumed *M* and *m* regulatory genes are expressed alternatively during development, in a manner similar to that discovered for the genes producing immunoglobulins (Burnet, 1969). The *m* alleles are activated only during the embryonic life when the lymphoid cells are differentiating from the common germinal line of cells, at other times the dominant *M* alleles are active. This would produce during the embryonic growth a large number of mutant cells each with a distinct immunological specificity. The pattern of each of these cells is transmitted by somatic inheritance to all descendents giving rise to large numbers of clones of cells, each unique in immunological specificity. (A similar phase-sensitive mutability is suggested in the case of the *S* gene mutations in plants of *Lycopersicum peruvianum* where de Nettancourt and Ecochard (1969) reported the generation of a new *S* allele "in sudden waves and at very high frequencies".) If the proposed mechanism

for the explosive origin of the antibody sequences is true, the differential resistance to infection among individuals may, to a certain extent, be a function of (1) the relative preponderance of one or the other form (*M*, *m*) of these regulatory genes, and 2) variations in the specific phase physiology. It is suggested that this mechanism formed the primitive basis from which specialised adaptions of wide significance in development, differentiation, mutation and infection may have evolved in the higher organisms.

7. Environmentally-Induced Heritable Variation and the Synthetic Theory of Evolution

One of the fundamental tenets of the Synthetic Theory of Evolution is the acceptance of the thesis of evolution by natural selection of *random, spontaneous,* heredi ary changes. The theory does not accept the idea that "directed" hereditary changes may be provoked by changed environment; that is, under certain favourable conditions mutations may be "directed" and not be entirely at random.

Earlier claims of environmentally directed mutation have all been dismissed as disguised notions of Lamarckism and, in any case, most of these could be equally well explained on the basis of the concept of selection pressure embodied in the Synthetic Theory (Stebbins, 1966). These include instances of hereditary changes of bacteria (Dean and Hinshelwood, 1957), hereditary adaptations of the nematode *Caenorhabditis elegans* to high temperature (Brun, 1965), resistance to insecticides by insects, and hereditary adaptation of the monophagous forms of insects from one host to other species (and other genera) (Andrewartha and Birch, 1954). Certain recent results, however, compel us to reconsider this important aspect of the Synthetic Theory, that "selection acts on *random* variation".

It is well known that heritable changes can be acquired through the application of ionising radiations and mutagenic chemicals, and these seem to point towards specificity of mutagens by way of secondary interactions, involving cytoplasmic factors, preceding or following the actual mutation of the DNA material (Auerbach, 1967). However, instances have appeared within this decade which demonstrate convincingly that the acquiring of heritable characters is not conditioned entirely by unique combinations of rare genetic and environmental factors. It can occur rather on a mundane level requiring no particularly rare situation. The pioneering work of Durrant (1962) and Evans, Durrant and Rees (1966) in *Linum usitatissimum* (flax), and Hill (1967) in *Nicotiana rustica* has shown that certain strains (plastic genotypes) of these species can acquire large heritable changes, for example in plant size or flowering time, according to the nutrients (combinations of N, P and K fertilizers) supplied. The two extreme, induced types in flax and *N. rustica* have been shown to be constant over a number of generations under the conditions of these experiments, irrespective of the nutritional environment. The association of these changes with alterations in the hereditary material has been shown by hybridization and inheritance studies of the extreme forms in both species, as well as by studies of nuclear volumes, total dry mass of nuclei, and photometric estimates and chemical analysis of nuclear DNA in flax. The nuclear data in flax are consistently and significantly higher in the larger form, lower in the shorter form and intermediate in the plastic forms.

There is no doubt at all about the genetic nature of the acquired changes — at least in the two plant species studied. We are, however, still very much in the dark regarding the genetic mechanism involved. The following points stand out consistently from the detailed studies of flax and *N. rustica*: —

1) Only certain strains of the two inbreeding species studied show induced heritable changes (plastic genotypes).

2) The changes are of quantitative nature.

3) The F_1 hybrids between the two extreme forms, large (L) and small (S), of the induced stable genotrophs are intermediate, variable and plastic.

4) The F_1 hybrids do not show complementation, and there are no pronounced reciprocal differences.

5) The presumed hereditary material involved in this phenomenon appears to be randomly distributed in the genome.

Regulatory genes provide a mechanism for biochemical differentiation of cells of identical genotypes. They may, therefore, provide the key not only to developmental processes but also to their temporary or permanent hereditary modifications. If the activity and mutability of structural genes are under the control of specific regulatory genes as is already suggested from the foregoing discussion, and if regulatory genes directly or indirectly are amenable to modifications through the intervention of certain specific cytoplasmic molecules or physiological stresses, then a way is open to the environmental induction of heritable changes. The above observations in flax and *N. rustica* can be explained on the basis of the present model with the following assumptions:

A. The induced changes are brought about by the action of the environment on the regulatory genes concerned with the specific characteristics involved. There are several such regulatory loci with alleles having dominance relationships. Only those plants are amenable to induction which are homozygous recessive for a considerable proportion of these loci. Thus only inbred plants would be exposed to such variation, and among these only certain strains would be amenable to induction.

B. The induction of heritable changes is associated with multiplication of these genes through duplication in the case of large genotrophs (L), and with

deletions of these genes in the case of small genotrophs (S). In this connection, three recent discoveries are significant. 1) Van't Hof and Sparrow (1963) have shown that among several species of higher plants a positive correlation exists between DNA content of the nucleus and the minimum time required for the mitotic cycle. 2) Callan (1963, 1967) and others (Ritossa and Spiegelman, 1965; Whitehouse, 1967) have provided evidence suggesting that each gene may be duplicated many times in a consecutive linear series within one DNA molecule and that these duplicate "slave" genes may be detached from, and attached to, the "master copy" gene at appropriate times during the nuclear cycle. Thus a mechanical basis for duplication and deletion of regulatory genes may already exist in the cell. 3) Kedes and Birnstiel (1971) have reported that the genome of sea urchin *Psammechinus milaris* contains many copies of the genes which specify histones. The role of histone proteins as repressors of gene action in higher organisms has been suggested from the work of Bonner and others (Bonner, 1965).

C. In the hybrids between L and S forms, the extreme dissimilarity of different homologous chromosomes, owing to duplications in one set and deletions in the other, not only gives rise to intermediate, plastic F_1 phenotypes, but also causes a certain amount of chromosomal instability which produces increased F_1 variation.

D. There are possibly only a few classes of regulatory genes, each class as a whole being susceptible to certain specific kinds of mutagenic stimuli.

(The above hypothesis regarding the mechanism involved in the induction of acquired heritable changes would suggest the possibility that a study of histones in the two genotrophs of *Linum* and *Nicotiana* might be rewarding.) The present hypothesis would further strengthen the role of inbreeding in increasing the rate of origin of genetic variation in higher organisms. Moreover, since such changes could be induced simultaneously in a number of independent organisms and would be generally specific to certain stimuli, they could be considered "directed" changes.

In a discussion of directability or otherwise of mutation, what concerns us ultimately is the net viable product of the hereditary change. From the foregoing discussion it cannot be denied that under certain environmental conditions, internal as well as external, organisms do acquire heritable changes and these may in a general way be specific or "directed". When the physiological pathway involved is relatively simple and direct the result may be predictable and understandable. A very common example is the cytological and associated genetical effect of colchicine treatment in plants producing polyploids (Eigsti and Dustin Jr., 1955). From this point of view, randomness of mutations may simply be a function of our ignorance of the underlying complexity of environmental and genetic interactions which bring them about. *Be this as it may, it is not implied here that environmentally acquired hereditary changes are necessarily "directed" in the sense we often expect them to be under the Lamarckian concept, that is in relation to the adaptiveness of the organism. They are "directed" in their own complex, inherent ways, the results of which may or may not include the physiological pathways yielding the Lamarckian end product. What is suggested here is that an environment containing a specific area of mutagenic potential, including effect(s) advantageous to the organism, might result in "directed" changes of developmental and evolutionary significance.* Viewed in this light, "canalization" (Grant, 1958; Stebbins, 1960, 1968; Waddington 1961, 1968) and "channelling" (Pandey, 1966, 1969c) assume an even greater potential for evolution. It is conceivable that some of the paleontological evidence of "bursts" of evolution in plants and animals, discussed by Davitashvili (1969) and others (House, 1963), may have their origin in this type of environmentally provoked "directed" changes. In this context, the present model underlines the significance of the regulatory genes, and of the breeding behaviour of the organism.

In conclusion, the model provides a self-regulated evolutionary balance between breeding system and genetic variability. The mechanisms controlling recombination and breeding system are thus extremely refined, being both complementary and interdependent; the two work together to conserve and release variability, and each adjusts and is adjusted by the other. The characteristic epistatic and dominance relationships of the regulatory genetic elements, their integration with the sexual mechanism and, probably, with development, and their hierarchical nature of flexibility, may bear testimony to the evolutionary complexity and antiquity of the system.

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