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Generation of Multiple Genetic Specificities: Origin of Genetic Polymorphism through Gene Regulation

Kamla K. Pandey

Genetics Unit, Grasslands Division, D.S.I.R., Palmerston North (New Zealand)

Summary. Based on results of mutation studies in the fungus *Schizophyllum commune*, a new mechanism of the origin of genetic polymorphism is proposed. This may explain the intractable problems of the rise of multiple allelism controlling incompatibility in plants and the wide array of antibody diversity controlling immunity reaction in animals.

Introduction

There are two intractable problems in modern genetics which have attracted wide attention in recent years, one concerning plants, the other animals. The gene controlling incompatibility in plants has literally hundreds of highly specific alleles in nature but paradoxically, all attempts to produce new alleles through mutagenesis have failed (Lewis 1954; Pandey 1956, 1965; Raper, J.R. and Raper, C.A. 1973; Stamberg and Koltin 1973; Ockendon 1974; de Nettancourt *et al.* 1975). Vertebrate animals appear to be capable of synthesizing an amazing diversity of antibody sequences, each presumably determined by a different antibody gene, but an understanding of how these genes arise is still uncertain (Dreyer and Bennett 1965; Burnet 1969; Smithies 1973; Smith 1973). Mutation and intra-locus recombination, the two well-known mechanisms by which new alleles arise, can not explain these problems. Here I propose a mode of origin of genetic polymorphism which may explain both of these phenomena.

Self-Incompatibility Genes in Plants

Complex self-incompatibility genes controlling breeding behaviour occur widely in the plant kingdom including flowering plants and fungi. Commonly, these genes have extensive series of alleles (Table 1). The consistent failure of attempts to produce new alleles experimentally, by mutagenesis and by intralocus recombination, in both higher and lower plants gave rise to a crop of theories to explain this phenomenon (Denward 1963; Pandey 1967, 1970, 1972; de Nettancourt 1972; de Nettancourt *et al.* 1975; Stamberg and Koltin 1973; Raper, J.R. and Raper, C.A. 1973). None of them, however, were supported by any direct evidence from incompatibility studies. The observations suggest that incompatibility allelism may depend on a third alternative mechanism for producing genetic polymorphism. Clues to the nature of such a mechanism have recently emerged from mutation studies in *Schizophyllum commune* (Raper, J.R. and Raper, C.A. 1973; Raper, C.A. and Raper J.R. 1973). In this basidiomycete fungus compatibility between haploid partners is controlled by 4 loci linked in two independently assorting pairs designated the A and B factors. Within each factor the two linked loci, α and β, each posses a series of alleles, and any given combination of α and β alleles determines a unique factor phenotype. Both factor phenotypes must be different for full compatibility. With only one factor in common between the partners (e.g. B common, incompatible, and A different, compatible) factor-specific incomplete morphogenetic developments occur (Fig.1).

In the study by J.R. Raper and C.A. Raper (1973) mutations in the β locus of the B factor were of two types: (1) Primary-universally compatible, lacking specificity, with B factor determination permanently switched on - i.e. constitutive for B morphogenetic genes ("B-on" phenotype). (2) Secondary - arising from primary mutants but 1000 times more frequent; however only 10 % of these involved the B_β locus, others occurred in genes not connected with the incompatibility loci and scattered in the genome. Phenotypic characters determined by "B-on" activity suppressed but giving rise to varying "B-off" phenotypes. All mutants self- and inter-incompatible.

Combinations of primary with secondary mutations gave the "B-on" phenotype, i.e. full complemen-

Table 1. Number of observed or estimated alleles in populations of different species of flowering plants

Species	Population size	S Alleles: Observed number	S Alleles: Estimated total	Author
Trifolium pratense	24	41	171	Williams 1947
	20	37	308	Bateman 1947
T. pratense			150 - 175	Whitehouse 1950
T. repens			75 - 175	
Oenothera organensis	< 500	45		Emerson 1939; Wright 1964
Raphanus raphanistrum	45	9	25 - 34	Sampson 1967
Iberis amara	52		at least 22	Bateman 1954
Brassica oleracea				
var. *gemmifera*	~ 500	19	41 - 70	Ockendon 1974
var. *acephala*		34		Thompson 1968

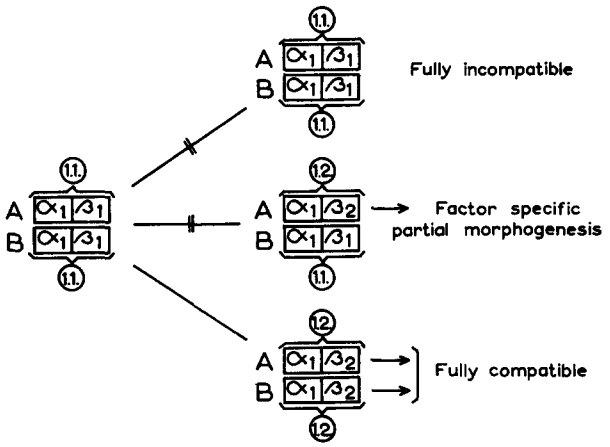

Fig. 1. Genetic control of incompatibility reaction in *Schizophyllum commune*. Circles represent factor phenotypes each determined by two component locus genotypes

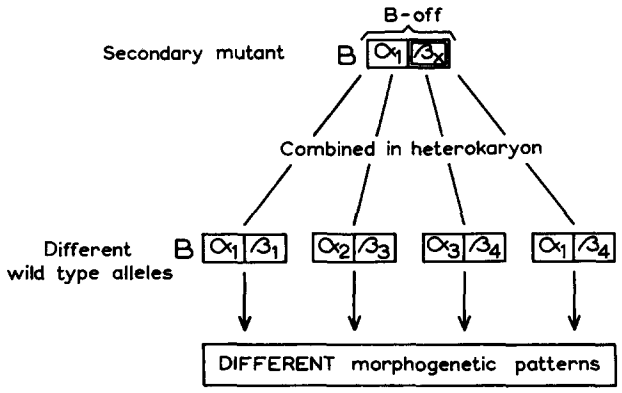

Fig. 2. Heterokaryotic interactions of a secondary B_β mutant in combination with different wild type alleles

tation by the primary mutant, but occasionally partial complementation was also observed, as in combination with B-always off type of secondary mutation. In the latter case the complementation by the primary mutant was only with respect to fusion of hook-cells (Raper, C.A. and Raper, J.R. 1973). Combinations of secondary mutants with wild-type alleles produced varying morphogenetic incompatibility patterns indicative of partial complementation. Significantly, however, each wild-type allele combined with the same secondary mutant produced a distinctive developmental pattern (Fig. 2). This was surprising since all normal wild-type alleles are considered equivalent in terms of incompatibility physiology and sexual morphogenesis.

All previous models for control of sexual interaction are inadequate to account for the range and behaviour of these B factor mutations (Stamberg and Koltin 1973; Raper, J.R. and Raper, C.A. 1973). An alternative model is proposed below which not only accounts for the behaviour of these mutants but also suggests a possible mechanism for the origin of new alleles at a complex locus such as the incompatibility "gene".

Proposed Model for the Mating Complex in *S. commune* (Fig. 3)

(1) The regulatory component(s), termed here 'Co-ordinator Gene', contains at least two functionally and

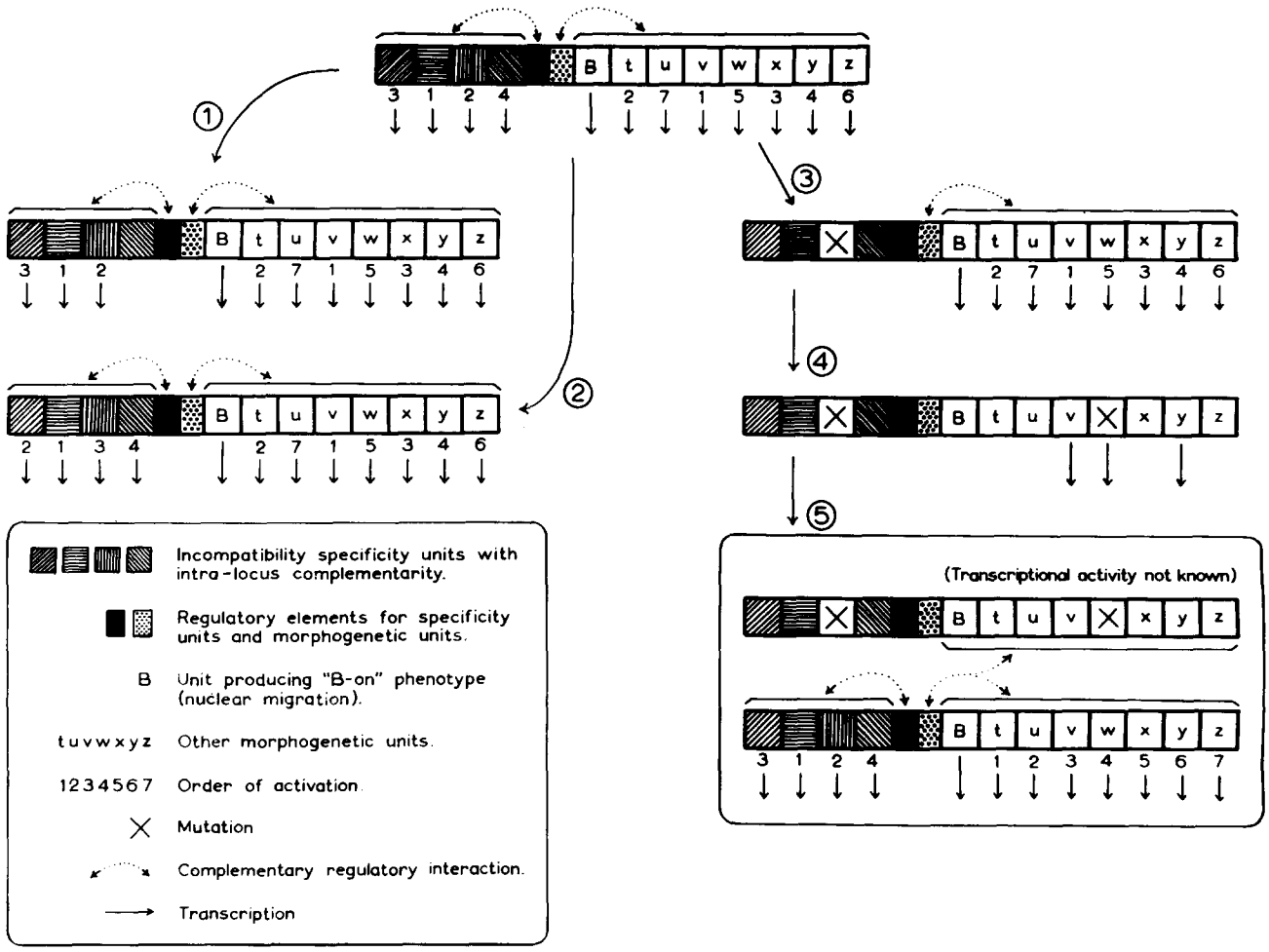

Fig.3. A proposed genetic model for control of sexual interactions in *Schizophyllum commune*, elucidating behaviour of mutants (steps 3-5) and mode of origin of new incompatibility specificities (steps 1-2). For detailed explanation see text

mutationally independent units responsible for two-way complementary interaction with (a) the specificity genes, and (b) the genes for sexual morphogenesis. However, it acts as a single receptor site for physiologic signals.

(2) Complementary regulatory interaction can be upset by changes in any of these three classes of genes (e.g. deletions or point mutations). Thus, presumably primary mutations affect mainly the specificity genes causing breakdown of interaction (1a) above, and resulting in a total lack of incompatibility specificity. The morphogenetic genes become constitutively switched on in the absence of incompatibility requirements, and since interaction (1b) above remains intact their expression goes to completion (Fig.3(3)). Secondary mutations presumably affect mainly the morphogenetic genes causing loss of interaction (1b), producing "B-off" phenotype and giving haphazard morphogenetic expression (Fig.3(4)). The possibility that the B secondary mutants may principally involve the co-ordinator gene is not ruled out but, for a number of reasons, is considered unlikely. The most significant factor favouring the present view is that a mutation that can be mimicked by so many independent genes in the background cannot be involved with a gene as specific as the co-ordinator gene.

(3) It is further proposed that when the interactions of the co-ordinator gene with the specificity genes on the one hand and the morphogenetic genes on the other take place normally it results in the expression of the morphogenetic genes in a physiological sequence regardless of their physical linkage relations - i.e. order of activity and order of linkage are independent. If physical order of linkage is inconsequen-

tial, random sequences may have accumulated in nature. Thus, in different wild-type alleles, the morphogenetic elements may be variously linked but normal regulatory interaction controls their expression in the same physiological order.

(4) To account for the anomalous behaviour of secondary mutants in combination with different wild-type alleles, it is suggested that a type of partial complementation occurs which allows expression of the morphogenetic elements of the wild-type allele according to their physical order of linkage rather than their normal physiological sequence (Fig.3(5)). Such expression in the wrong physiological sequence may result in breakdown of complementation at any one of the different stages of sexual morphogenesis. Thus the variable patterns of cross-compatibility behaviour produced by combining individual secondary mutants with various wild-type alleles reflect the different linkage relationships of the morphogenetic genes in the wild-type alleles.

Model for the Origin of Incompatibility Alleles in Plants

(1) If there is a co-ordinator gene in the complex controlling which morphogenetic genes are to be expressed and in what precise complementary physiological sequence they will be activated, then the same may be true for the specificity genes which act in a complementary manner to produce a single allelic specificity. Changes in the on/off state and order of expression of a relatively small number of specificity genes could produce large numbers of distinct incompatibility alleles (Fig.3(1) and (2)). (In the S locus model for homomorphic flowering plants there would be no apparent morphogenetic genes but there are likely to be genes controlling general aspects of the physiology of reproduction.)

(2) The stability of the co-ordinator gene which governs the precise complementary activation of the specificity genes must itself be under strict control and must have a high buffering ability so that once a new allelic state has been produced it is stable over a relatively wide range of genetic and environmental situations. The condition causing a new specificity to be switched on may be a function of equilibrium between the incompatibility complex and the cytoplasmic milieu in which it operates, the latter being governed by the physiological specificity of the tissue and the general genetic and environmental background. Presumably the most conducive background condition for stability of incompatibility alleles is given by a relatively high degree of heterozygosity, as postulated for control of recombination in specific regions (Pandey 1972). If this is the case, (i) inbreeding may be helpful in the production of new incompatibility alleles, as has been shown in *Trifolium*, *Lycopersicon* and *Nicotiana* (Denward 1963; de Nettancourt and Ecochard 1969; Pandey 1970a; Anderson *et al.* 1974), and (ii) the original allele might occasionally be retrieved by a return to approximately the original polygenic background after backcrossing, as has been found in *Lycopersicon* (de Nettancourt and Ecochard 1969). A similar effect may also account for reactivation of inactivated S loci on transfer to a new genetic background, as has been suggested to occur in *Solanum* and *Lycopersicon* (Pandey 1957, 1970b; Martin 1967).

The model also allows the possibility, suggested by the recent work of van Gastel and de Nettancourt (1975) that in a gametophytic system while a diploid plant may produce only two kinds of male specificity, a pollen grain bearing one or the other, on the female side the stylar tissue may bear more than two different specificities.

Interestingly, the model throws light on how the complementary incompatibility system, in which alleles of two or more different series co-operate to produce a single specificity, might function. In this case a single co-ordinator gene may control the activity of specificity genes belonging to two or more complexes. It has been suggested that complementation may occur at the transcription level. Thus, in Gramineae, the two groups of linked specificity genes characterizing alleles of the S and Z loci may be transcribed together in a controlled sequence to produce one final molecule (Pandey in press).

General Model for Genetic Polymorphism in Eukaryotes

Basic to the hypothesis outlined above is the concept of complementation, understood in the broadest functional sense, and not in the restricted sense of Benzer's definition (1955) (Fig.4). Correct stepwise development requires complementary interactions between genes, the modes of action of which may vary

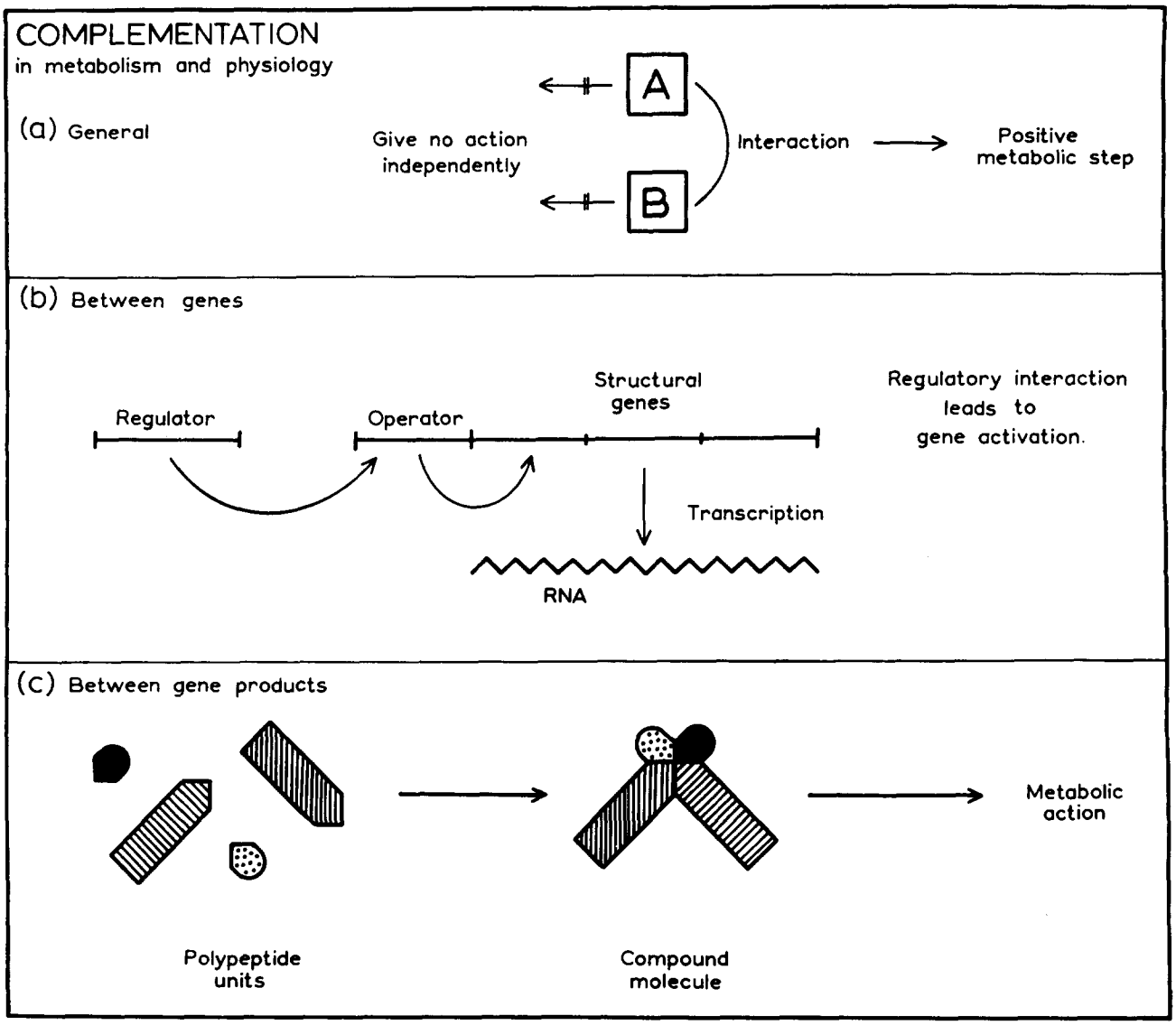

Fig.4. Examples of complementation conceived here in the broadest functional sense

greatly between systems. For example, a number of genes controlling the same character may be either linked together in a block or gene cluster, or they may be located on different chromosomes or chromosome arms. If they are located in a cluster they may be either serially linked in the complementary order of their activation, or they may be linked haphazardly but their activation in the complementary functional sequence may be controlled by a co-ordinator gene. Again, genes in the cluster may have either arisen through tandem duplication and subsequent differentiation or, they may have been brought together through convergence, as in adaptive gene combinations usually called supergenes (Fig.5). The original tandem duplication may involve either a single gene, or a chromosome segment (Darlington 1956; Mather 1950; Ford 1964; Ohno 1970, 1973). If the genes are unlinked they may still function either as a 'serial system' with their order of activation being controlled by the developmental sequence (Grant 1975), or as a combination of linked and unlinked systems in which different genes are switched on or off in a complex reticulate development through the aid of a battery of co-ordinator genes. All these different types of gene associations and arrangements possibly require different kinds of complementary mechanisms to achieve the required functional end.

Complementation mechanisms may also vary according to the level at which complementation occurs: (i) gene transcription, (ii) messenger RNA, (iii)

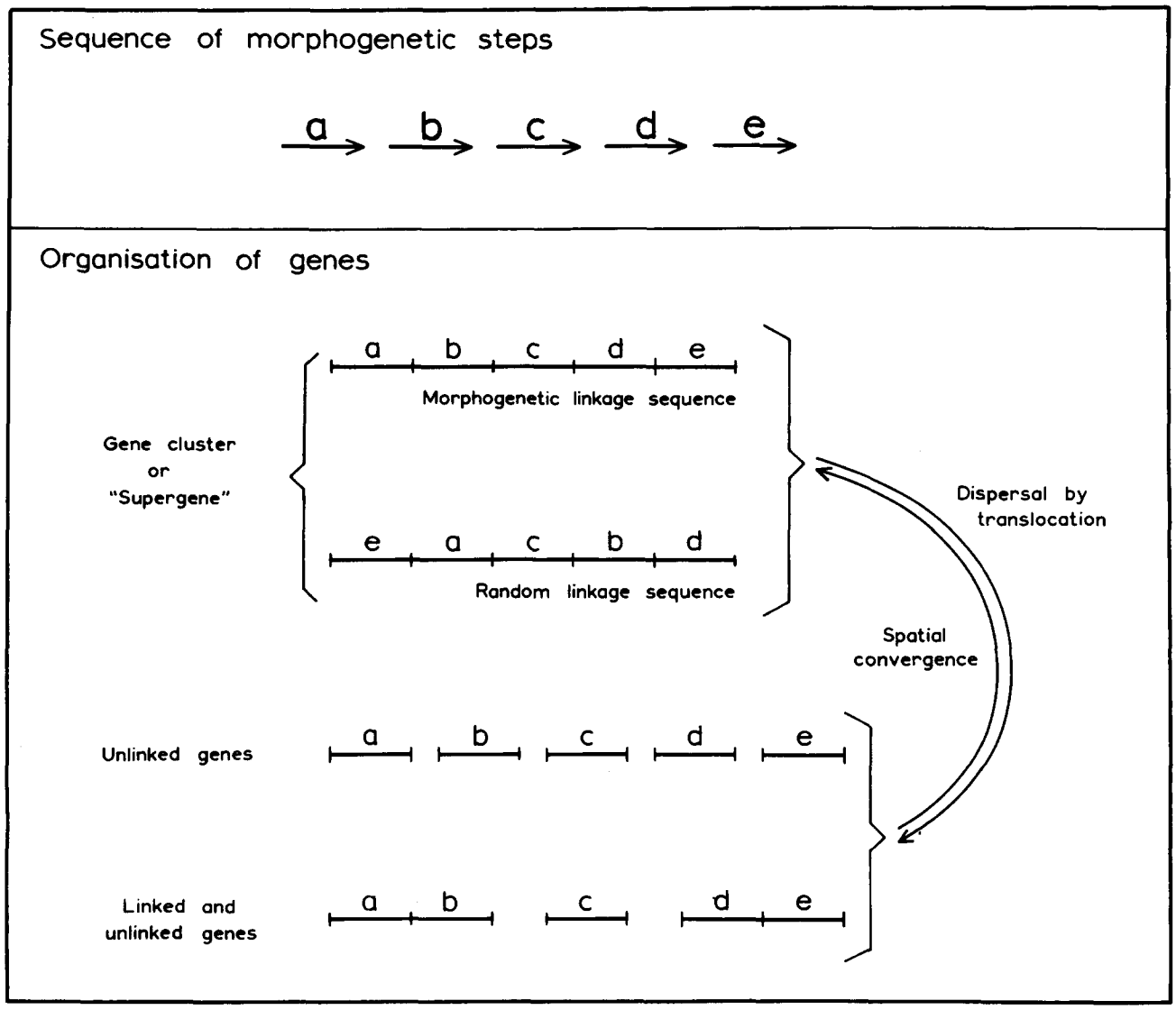

Fig.5. An example of evolutionary relationships in complementary units

gene translation, or (iv) gene product (polypeptide). Examples of trans complementation at the polypeptide level and that of cis complementation at the gene transcription level are well known (Fincham 1966, 1972). Recently Gally and Edelman (1972) have proposed a model of complementation at the gene transcription level by which spatially separated elements of a group of genes in tandem array could be spliced together through an intervention of loops. The spectrum of genetic organization in prokaryotes and eukaryotes would of necessity be different in this connection.

Changes in the site of these integrated genes may make the respective complementary mechanism inappropriate and may produce disorders of a type called 'position effect' by Goldschmidt (1955). The relatively rare condition where a mutant gene is dominant over the wild-type allele, may be due to the mutation altering the balance of the complementary mechanism to cause preferential expression of the mutant gene. Survival of the essential complementation mechanisms must be ensured through mechanisms restricting crossing-over which would otherwise destroy them. A possible extreme case of preservation of such linked genes is obtained from the study of banding patterns of chromosomes which shows that the original linear order of the x-linked genes was conserved to a remarkable degree during the whole of mammalian evolution (Ohno 1973). Another possibly extreme example where preservation of a system of complementation may be significant concerns

functional repression of one chromosome, again in relation to the x-chromosome (Lyon 1971). Within the family of linked genes on the x-chromosome complementation involving genes on the repressed allosome may not occur and complementation concerning genes located on the functional x-chromosome might overwhelmingly involve genes located on autosomes. The situation may be significant in relation to sex determination.

Complementation may be significant in the understanding of another paradoxical situation in animals: DNA constancy in the face of karyotype instability. Thus, although the chromosome number varies by more than one order of magnitude the amount of DNA per cell is very similar (Atkin 1965). The karyotypic difference between species may be related to the specialization of mechanisms of complementation governing selective gene activity.

Inasmuch as complementation may be a general feature of complex forms of development, characteristic of all eukaryotes, the hypothesis may have a broader application to differentiation in general. Observations have been made in several areas of research which are in general agreement with the hypothesis - i.e. the production of genetic polymorphism by variable complementary interaction between a set of genes and a co-ordinator gene, which controls their activity and sequence of expression, and which in turn is responsive to multiple physiological thresholds generated in development through the interaction of environment and the general genetic background, in a way similar to that proposed by Britten and Davidson (1971) involving 'sensor' genes. For example:

(1) Evolution of monoecism in flowering plants: While in an overwhelming proportion of plants determination of mating types is achieved through genetic segregation, in a tiny minority, the monoecious plants, it is determined by genetic regulation and is integrated with the process of differentiation. In these plants, the female and male flower forms are expressions of the same genotype. Natural examples of conversion of dioecism to monoecism (Lloyd 1975) show how the elements of both systems may coexist in the same species, one system operating while the other is suppressed.

(2) The appearance of different allelic forms of the same enzyme in different tissues of an individual (Harris 1969), which may be termed 'gene typing', may result from differential qualitative and sequential transcription of component genes in response to the differing internal environment of different tissues.

(3) There is much reported evidence for a similar mode of origin of diversity of immunologically specific cells in animals (Cooper 1975). Each antibody molecule usually consists of two pairs of polypeptide chains, light chains which can be either of two types, kappa or lambda, and heavy chains which can be of five types. These are coded by three complexes of closely linked genes: one complex each for kappa, lambda, and heavy chains. Each complex consists of a family of V genes coding for V (variable) regions and one or more C genes coding for C (constant) regions. These clusters of V and C genes are spatially separated although nearby on the same chromosome (Dreyer and Bennett 1965; Burnet 1969; Smithies 1970, 1973; Smith 1973). A closely linked cluster is formed by genes for various classes of heavy chains but the genes for kappa and lambda class of light chains are not linked to that cluster. Each immunoglobulin molecule is thus not coded by a complete discreet VC germ line gene (Dreyer and Bennett 1965; Smithies 1973). However, there is evidence from sequence analysis of immunoglobulin light chain messenger RNA showing that there is one molecule, and not two separate molecules, coding for the V and C regions of the light chain (Milstein *et al.* 1974). Apparently complementation at the transcription level occurs between spatially separated elements of a group of genes in tandem array (possibly through splicing together of genes on the Gally and Edelman model), and complementation between unlinked as well as linked genes occurs to produce the complete antibody molecule. Further, there are indications that in each cell only certain genes of a cluster are expressed while others remain 'silent', and that in different types of cells different components of the same cluster are activated (Cooper and Lawton III, 1975). Thus while there are no germ line operons coding for each type of immunoglobulin, all genes required to produce them are nevertheless already present in the germ line, and polymorphism may be generated through environmentally induced random

and non-random inactivation and qualitative differential expression of the structural genetic segments involved. Immunoglobulin specificity determination may, therefore, mainly occur over a steep environmental gradient occurring in the early lymphoid organs in the foetus (Decker and Sercarz 1974).

Differential regulation of a spectrum of subunits to produce polymorphism at complex loci is an attractive hypothesis for long term evolution. Since it does not involve mutation or recombination there is no loss of established genetic material. A high degree of sophisticated variation can be maintained under the control of basic regulatory elements which are sensitive to the innumerable background thresholds produced by selection, hybridization and inbreeding. In this scheme an allele, or specific mode of development, may be recreated after a lapse of millions of years, when the species concerned may have given rise to different genera or even families. Indeed, the unique parallelism of multiallelic incompatibility systems throughout the plant kingdom may bear testimony to the inherent potential and stability of this mode of genetic polymorphism. It may be one of the mechanisms by which essential life processes or molecules, once evolved, can be maintained and adapted to suit innumerable environments and developmental patterns in a large body of organisms comprising a whole kingdom.

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Dr. Kamla K. Pandey
Genetics Unit,
Grasslands Division,
D.S.I.R.,
Palmerston North (New Zealand)