ARTIFICIAL SYNTHESIS OF NATURALLY-OCCURRING PLANT SPECIES—ITS BEARING ON PLANT TAXONOMY

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The living world is characterised by variation. Taxonomically we may recognise two broad trends from a survey of the present-day flora. There is, on the one hand, evidence of remarkable evolutionary stability as for instance in the blue green algae. Depending upon the environment in which it is placed a single genotype of a blue green alga can assume forms so diverse that taxonomists have ranked them as separate species or genera (Crow, 1924). There is perhaps no other organism in which so few genotypes have spanned such a diverse habitat. On the other hand, we have in the case of the Angiosperms variation in the extreme form, giving rise to the entire gamut of hierarchy of taxa (orders, families, genera and species). Evolutionary data suggest that the Angiosperms as a group are adapted to primarily tropical conditions. But as many as forty out of the three hundred and odd families are narrowly confined to the temperate and the dry regions marginal to the tropics. It is in the study of this variation that the taxonomist derives relationships between forms depending upon whether or not intergrades between distinct types are present. It is in this connection the question should be posed. Do taxa arise abruptly? Evidence that they do is from two sources, viz., the abrupt appearance of taxa in the fossil records and discontinuity of characters and forms of the Angiosperms that are extant. Even if we exclude, as Simpson (1960) does, the fossil gaps as due to sampling bias, there appears to be no explanation why graded transition between radially symmetric and zygomorphic flowers, between separate corolla and united petals, and between the head of the compositae inflorescence and the conventional type of flower, does not occur.

The question whether 'abrupt speciation' or 'graded transition' has been the major factor of evolution is not necessarily answered exclusively by either one of the alternatives. Inferences have largely been deductive so far since reproduction of evolutionary changes in experiments would require periods far greater than the life span of the investigators. There are, however, two fortunate exceptions to this and they are, experimentally induced mutations and alloploidy. These will be discussed in some detail here in as far as it concerns the role of the taxonomists in evolving a system of classification based on natural affinities. It is proposed to show that while the general trend of evolutionary advance may be due to the operation of natural selection on randomly varying population resulting in the slow accumulation of small changes, there could be sudden spurts of evolutionary activity resulting in taxa which show no graded relationship among themselves. The two genetic forces that are capable of bringing this about are mutation and polyploidy. Illustrations are mainly restricted to the work of the author and his collaborators at the Indian Agricultural Research Institute.

Let us begin with the example of wheat. Members of the genus *Triticum* belong to a polyploid series with $2n = 14$, 28 and 42 chromosomes. Cytogenetic studies of Kihara and Lilienfeld (1949), McFadden and Sears (1946), Sarkar and Stebbins (1956), and Riley, Unrau and Chapman (1958) have established that the tetraploid ($2n = 28$) and hexaploid ($2n = 42$) species arose by chromosome doubling in hybrids between einkorn wheat ($2n = 14$) and *Aegilops speltoides* ($2n = 14$) and emmer ($2n = 28$) and *Aegilops squarrosa* ($2n = 14$) respectively. It is the hexaploid species that concern us now. This group consists of the following species :

i. FREE-THRESHING :

    T. aestivum L.
    T. sphaerococcum Perc.
    T. compactum Host
    T. amplissifolium Zhuk.

ii. NON-FREE-THRESHING :

    T. spelta L.
    T. macha Dek. et Men.
    T. vavilovii Jakubz.
    T. zhukovskii Men. et Er.

Each of the above species possesses a whole constellation of morphological traits distinct enough for the systematist to accord specific status to each group. But a remarkable genetic feature of this group is that the key characters that separate most of the hexaploid *Triticum* species are governed by only one

or two genes. Mackey (1954) has shown that *T aestivum* and *T. spelta* differ by one major gene, *Q*, situated on the long arm of chromosome 5A (nomenclature of Sears, 1959). Likewise *T. sphaerococcum* and *T. compactum* are both separated from *T aestivum* and *T. spelta* differ by one major gene, *Q*, 3D in the case of the former and *C* located on 2D in that of the latter. Thus the *sphaerococcum* and the *compactum* types could have arisen only after the origin of the first hexaploid *Triticum*. The Chinese wheat *T amplissifolium* has spherical grains combined with the compactoid ears of the *inflatum* type. Singh, Pal and Anderson (1957) have shown that *T. vavilovii* differs from *T aestivum* only in two genes. Zhukovskij (1962, Hudson's translation) believes this species to have 'definitely originated by mutation', a suggestion which has been experimentally proved by Prabhakara Rao and Swaminathan (1963).

What is the significance of these distinctive characters being the effect of one or two genes? Such manifold effects of a single gene might imply that the gene concerned affects one of the early determinative processes in the ontogeny common to the development of all the affected characters. Alternatively, the observed unit of recombination is in fact, a string of tightly linked genes, a "super gene" or a "switch gene" (Mather, 1961). If the latter, it should be possible to separate the different phenotypic effects of the 'super gene', a very desirable situation for the plant breeders. Of immediate concern to us are the genes *C*, *S* and *Q*. Previous analyses at regrouping hexaploid wheats by Schiemann (1948), Mackey (1954) or Sears (1959) have indicated the resolving power of conventional genetic analysis not to be high enough to assess the exact nature of these loci.

At the Division of Botany, Indian Agricultural Research Institute, my colleague, Dr. Swaminathan and his group, have attempted a mutational approach to the problem. Although the phylogenetic analysis of *Triticum* cannot be said to have been completed, considerable light has been thrown on this complex problem. A detailed account of this elegant piece of work has already been presented (Swaminathan, 1963) and I shall mention only such conclusions as are germane to the issue of the taxonomy of *Triticum*.

All the hexaploid species were treated with varying dosages of physical mutagens like X-rays, gamma rays, fast neutrons and thermal neutrons and of the chemical mutagen ethyl methane sulphonate (Swaminathan and Rao, 1960, and Rao, 1962). Mutants resembling *T. spelta*, *T. vavilovii*, *T compactum*, *T. sphaerococcum* and *T. amplissifolium* were isolated

in the progeny of *T. aestivum* treated with different doses of physical and chemical mutagens. Conversely, from the treated *T. sphaerococcum* mutants resembling *aestivum* were obtained, thus rendering the suggestion that *sphaerococcum* might have arisen through chromosome deletion (Ellerton, 1939) improbable. Likewise *aestivum*-like mutations also occurred when *compactum* was subjected to the mutagenic treatment. Forms resembling *T spelta* could be obtained from *T vavilovii* on mutagenic treatment.

Mutational analysis has also yielded fascinating results regarding the dominant role played by the gene *Q* responsible for the free-threshing habit. A gene of fundamental significance to the plant for its twin needs of self-propagation and self-protection, it ensures organised flower morphogenesis. By affecting cell shape and turgidity, it controls plant height, culm thickness, leaf surface, ear laxity and toughness. This gene shows not only a dosage effect but also epistatic action on other genes controlling characters like brittleness of the rachis, elongation of rachillae and spikelet sterility. Based on observations of association of all the different quantitative and qualitative effects arising from *Q* mutations, the complete speltoidy when the entire *Q* is lost, and partial losses leading to reduced expression of speltoidy, it has been suggested that the *Q* gene might consist of a series of repeats (Swaminathan, 1963). When *Q* gets replicated 3 or 4 times sub-compactoid and compactoid phenotypes appear. When it is present in a single dose speltoid characters are pre-eminent. The free-threshing effects are probably stabilised at the disomic level in the *vulgare* types. The concept of the nature of the *Q* gene as one of tandem repeats appears to explain most of the observations in the genetic and mutational analyses. We may visualise that a basic Q_1 allele present in *T. monococcum* has been transmitted to the tetraploid, *T carthlicum*, *T durum*, *T turgidum* and the hexaploids, *vulgare*, *compactum* and *sphaerococcum*.

An essentially similar nature of the '*S*' gene has also been inferred. The sphaerococcoid mutants in *vulgare* were of two types (Swaminathan, Jagadesan and Chopra, 1963) ; one allelic to the '*S*' locus of the subspecies *sphaerococcum* and the other like the mutants of Schmidt and Johnson (1963) non-allelic to the '*S*' of *sphaerococcum*. Probably at least two independent loci are capable of generating the *sphaerococcum* syndrome of characters.

With reference to the *C* locus, mutants with the *compactum* phenotype were not isolated from the treated *vulgare* while *vulgare* mutants were very common in the progenies of irradiated *compactum*. A

single gene difference between these mutants and compactum was also noticed. There does not appear to be any recombination within the C locus. The ear compactness of T macha has been shown to be different from the C locus of compactum and if the C locus is shown to be present in any tetraploid Triticum species then compactum could be phylogenetically older than vulgare. But the presence of the C locus in the D genome suggests the evolution of compactum from a pre-existing hexaploid.

To sum up, the mutational analysis of the phylogeny of the hexaploid wheats presents a picture in which the hexaploids vulgare, spelta, macha and zhukovskii are derived independently from pre-existing tetraploids. Compactum, sphaerococcum and vavilovii must have arisen from a pre-existing hexaploid. Particularly vavilovii must have evolved out of a speltoid form of vulgare. The remarkable feature of this analysis is that it brings out the role of mutations in evolution. Though the process of evolution through mutation is an extremely slow one, providing the raw material for the natural selection to act upon, occasionally jumps of larger quanta in the evolutionary scale are possible.

It is therefore reasonable to ask whether one would not be justified in grouping all the hexaploid species under one specific taxon. This has already been considered by Mackey (1954), Schiemann (1948, 1951) and Sears (1959). The mutational analyses discussed here lends further rationale for such grouping.

Another genetic mechanism by which abrupt engendering of taxa could be achieved is the stabilisation of inter-taxa hybrids by chromosome duplication, i.e., alloploidy. Fortunately the operation of this mechanism is capable of experimental proof and there is available in the literature a number of instances of artificial syntheses of alloploid species. Of these I shall refer only to those studies in detail by my colleagues at the Indian Agricultural Research Institute.

The existence of genomic alloploidy has been inferred in the Brassica group of plants by Morinaga (1934) mainly on the basis of cytological analysis of hybrids between natural digenomic tetraploids and elementary diploid species. The first experimental demonstration in this group was the artificial synthesis of Brassica juncea in 1941 by Ramanujam and Srinivasachar (1943) thereby providing proof of the genomic analysis of Morinaga (1934). The artificially synthesised hybrid not only resembled the naturally occurring B. juncea but also was compatible with the latter, giving rise to fertile hybrids. Like its natural

counterpart it was also self-compatible while the elementary species B. nigra and B. campestris are both self-incompatible. The morphological features that characterise B. juncea, viz., the non-amplexicaul stem leaves, introrse dehiscence of the inner whorl of anthers and seed coat characteristics could all be seen in the synthesised one and there could be no doubt that in this case the evolutionary process has been re-enacted in the laboratory.

Another interesting feature is the occurrence of self-compatible forms in the elementary species campestris itself. Genetic and cytogenetic analysis of the campestris group has led to the formulation of an hypothesis regarding the evolution of yellow sarson (Rajan, 1958). It was seen that genes for as many as four different characters concerned with pollination were located on the same chromosome showing close linkage. The self-compatible and the self-incompatible groups also differed by an inversion on one of the chromosomes. This inversion could act as a recombination suppressor and result in close linkage of the genes located in the inverted segment. It is postulated that a long distance dispersal of a self-incompatible type could have accumulated genes favouring self-pollination and recombination suppressing mechanisms like inversion could have given rise to the yellow sarson abruptly. It may be mentioned that early taxonomists had accorded the yellow sarson a specific status. An essentially similar mode of evolution but through disruptive selection rather than long distance dispersal has been proposed for the self-compatible forms of brown sarson which is ordinarily self-incompatible (Murthy and Mathur, 1963).

We may conclude from these studies that when a species colonizes new habitats it might achieve immediate fitness to the new environment through stabilized alloploidy or through mutations at individual loci. The former would naturally be more abrupt.

Another instance where the role of alloploidy in evolution of species has been demonstrated is in the case of Abelmoschus. The cultivated okra, A. esculentus, is characterised by its spatheceous, circumscissile and deciduous calyx which, fused with the stamens and corolla at the base, falls as one piece after anthesis. The demonstration of the alloploid nature of the cultivated okra was rendered possible by the isolation by Pal, Singh and Swarup (1952) of a new species, A. tuberculatus. The status of a new species was accorded to this material not only on the basis of its morphological features but also on crossability studies with other species like, esculentus, ficulneus and manihot, and on the fertility of the hybrids from these crosses. Further

cytogenetic analysis of this material by Joshi and Hardas (1953), and Hardas and Joshi (1954) has clearly demonstrated the alloploid nature of the cultivated okra. Out of the 65 haploid chromosomes of okra, 29 appear to be homologus with the 29 chromosomes of *A. tuberculatus*. This establishes the latter as one of the constituent genomes of okra. The identity of the other genome with 36 chromosomes has not been established and it appears that none of the species with 36 chromosomes studied so far is involved. On the other hand an amphiploid species has been synthesised using the new species *A. tuberculatus* and another species with 36 chromosomes, *A. ficulneus*. The resulting amphidiploid though chromosomally like *A. esculentus* has no morphological or cytological relationship with it. Unlike the case of *Brassica* the artificial synthesis has not resulted in the exact recreation of the natural species since one of the component species remains unknown. But we can consider the process to have been adequately demonstrated.

If in the case of *Brassica* a natural species has been resynthesised, and in that of okra its alloploidy demonstrated, in the case of *Sesamum* a new species has been synthesised which had not occurred in nature before. This is *Sesamum indicatum* (2n = 58) derived as an amphidiploid through colchicine treatment of the hybrid *S. orientale* (2n=26)×*S. prostratum* (2n=32) (Ramanujam, 1944).

It is axiomatic in taxonomy that relationship is due to the common origin of discontinuous and variable units. It is the endeavour of the geneticist to study the variations and seek to define the discontinuity in terms of cytology and genetics. The development of sterility barriers and discontinuities are far from thoroughly understood. From the observations presented in this paper the nature of the mechanisms of what the taxonomists call 'abrupt speciation' can be discerned albeit vaguely. Despite an ostensible wealth of information our knowledge concerning the effect of various biological phenomena on the dynamics of evolution is still fragmentary and limited. The contribution of experimental techniques such as have been described here towards a better understanding can indeed be very great.

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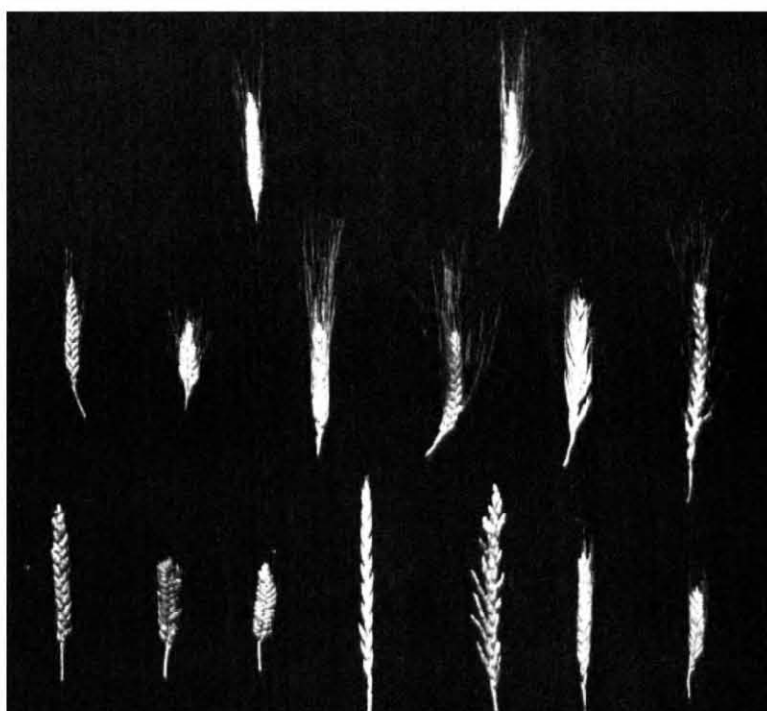

PLATE I

Ears of diploid, tetraploid and hexaploid species of *Triticum*.

Row-1. Left to right :   *T. monococcum, T. dicoccoides.*

Row-2.   „   „   *T. dicoccum, T. timopheevi, T. durum,*
T. turgidum, T. polonicum, T. persicum.

Row-3.   „   „   *T. aestivum, T. compactum, T. sphaero-coccum, T. spelta, T. vavilovii, T. macha, T. zhukovskii.*
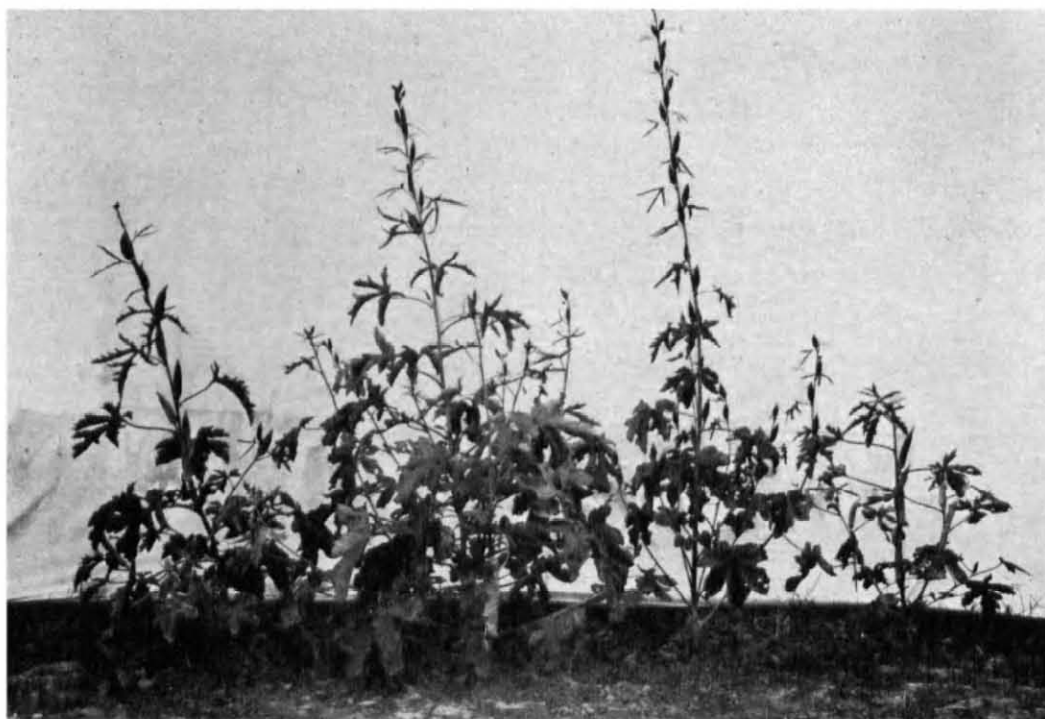
PLATE II

Species of *Abelmoschus*. From right to left :—*A. esculentus, A. tuberculatus,* the hybrid
A. esculentus✕*A. tuberculatus,* the artificial amphidiploid *A. esculentus*✕*A. tuberculatus.*

PLATE III

Brassica species : From left to right : *B. juncea, B. campestris, B. nigra.*