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Elimination of Heterozygosity and Efficiency of Genetic Systems

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Summary. One of the significant observations of recent years in the field of population genetics, highlighted by electrophoretic isozyme studies, is the presence of considerable heterozygosity within experimental and natural populations of highly inbred plants. This is found to be a general phenomenon, and is attributed to heterozygote advantage. In "Parthenogenetic Diploidy", where an organism develops without fertilization after doubling of the haploid egg nucleus, genetic heterozygosity is abolished altogether. Parthenogenetic diploidy, therefore, offers an excellent opportunity to examine the relevance of heterozygote advantage to the efficiency of genetic systems and maintenance of specific populations. In this paper, based on the study of comparative incompatibility behaviour in parthenogenetic diploids and parent plants, a hypothesis is proposed explaining the significance of persistent heterozygosity in inbred populations.

The role of heterozygosity in genetic systems can be long-term, evolutionary, through segregation and recombination, termed here SEGREGATIONAL heterozygosity; or immediate, developmental, through allelic "co-action" or interaction, INTEGRATED heterozygosity. It is proposed here that a certain degree of genetic heterozygosity of the INTEGRATED type is incorporated in the regulatory polygenic components of various genetic systems involved in an organism, and may be essential for the normal development characterising a physiological system, an ecotype, a species, or a higher group. INTEGRATED *heterozygosity is effective in overcoming the barriers of limited threshold regimes in physiological systems, and hence is particularly significant in extending the inherent plasticity in the physiological expression of genetic systems.*

1. Introduction

Isozyme studies of inbred populations in several species of flowering plants by Allard and associates (Allard, 1965, 1966; Allard, Jain and Workman, 1968; Allard, Kahler and Weir, 1972; Kannenberg and Allard, 1967; Marshall and Allard, 1970) and others (Srb, 1966) have shown that it is almost impossible to produce perfectly homozygous organisms irrespective of the number of generations of inbreeding. These workers found a remarkable degree of genetic variation within experimental and natural populations of highly inbred plants, and attributed the persistence of heterozygosity to heterozygote advantage (Fincham, 1972). Allard (1965) concluded that a "high degree of variability is essential to the survival of populations".

The fact that the occurrence of a considerable degree of heterozygosity is universal, in inbreeding as well as outbreeding plants, poses theoretical problems which have not been satisfactorily resolved. Is the notion of heterozygote advantage in terms of individual genes sufficient to explain the widespread occurrence of heterozygosity? Or, is there an additional, more basic reason underlying this phenomenon?

The role of heterozygosity in genetic systems can be longterm, evolutionary, through segregation and recombination, termed here SEGREGATIONAL heterozygosity; or immediate, developmental, through allelic "co-action" or interaction, INTEGRATED heterozygosity. Fig. 1 summarizes the suggested functions of heterozygosity in genetic systems.

I propose here that a certain degree of genetic heterozygosity of the INTEGRATED type is incorporated in the regulatory polygenic components of various genetic systems involved in an organism, and may be essential for the normal development of organisms characterising a physiological system, an ecotype, a species, or a higher group. INTEGRATED heterozygosity is effective in overcoming the barriers of limited threshold regimes in physiological systems. Hence, it is particularly significant in extending the inherent plasticity in the physiological expression of genetic systems to the widest range commensurate with the internal and external environments of the organism. INTEGRATED heterozygosity may, thus, be relevant to the well-being and survival of populations as well as individuals. In order to test this hypothesis in plants, perfectly homozygous individuals are required in a material which has a relatively well-understood specific genetic system. One way such individuals can be obtained is through parthenogenesis.

2. Incompatibility and Parthenogenetic Diploidy

Intra- and interspecific incompatibility in flowering plants is a well known genetically controlled phenomenon. In the genus *Nicotiana*, the normally self-incompatible species *N. forgetiana* has two kinds of plants, one whose styles accept the pollen of the sister self-compatible species *N. langsdorffii* and the other whose styles reject this pollen. In an experiment designed to overcome the incompatibility in the combination *N. forgetiana* × *N. langsdorffii*, irradiated pollen (700 r x-rays delivered at meiotic or

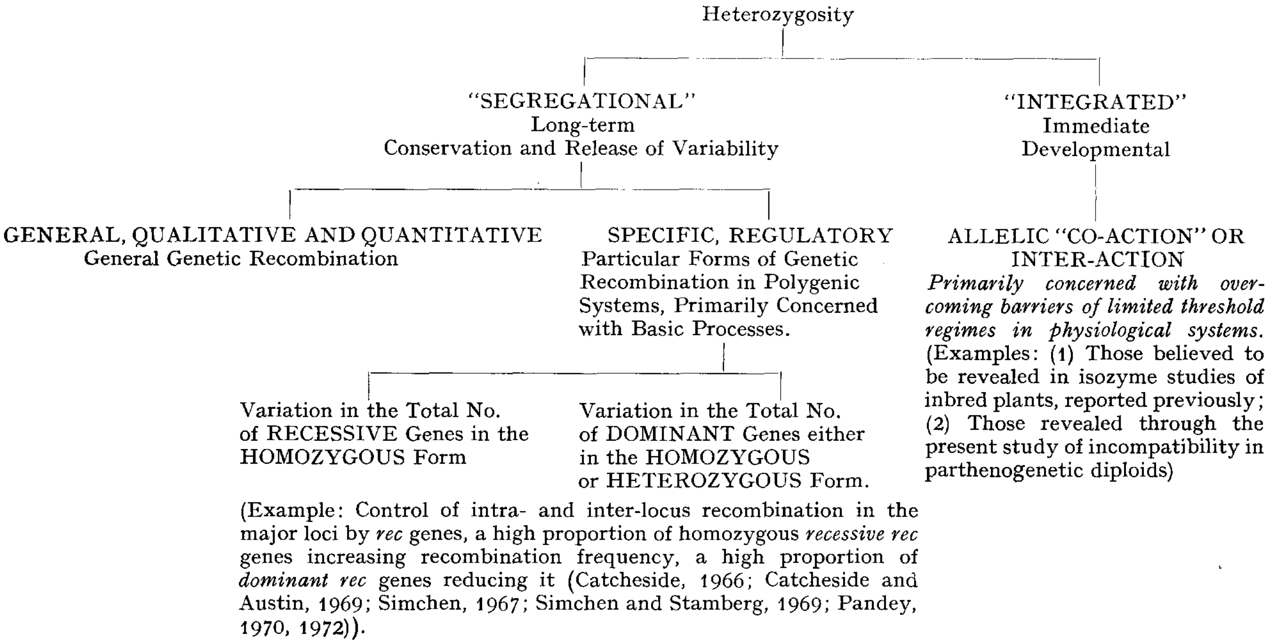

Fig. 1. Functions of heterozygosity in genetic systems

premeiotic stages of flower buds) of *N. langsdorffii* was used. From several hundred pollinations a few seeds were obtained, which eventually produced 8 plants. These plants which were fully fertile diploids were, however, not hybrids but, as evident from their morphological and floral characteristics, pure *N. forgetiana* produced presumably through parthenogenesis (egg doubling its chromosome number and developing into embryo without fertilization, termed here "Parthenogenetic Diploidy" to distinguish it from the phenomenon, believed not to be involved here, called "Diploid Parthenogenesis" in which the egg itself has the diploid chromosome number). This showed that no mutation occurred in the irradiated pollen of *N. langsdorffii* to break down incompatibility and allow fertilization. The details of this and certain other experiments in *Nicotiana* elucidating interspecific incompatibility will be given elsewhere. This report describes the origin of these maternal plants, and discusses their unexpected behaviour with regard to intraspecific self- and cross-incompatibility as effected by the drastic genetic change brought about by the total loss of heterozygosity.

♀ \ ♂	1	7	8	2	3	4	5	6	P
1	—	—	—	+	+	+	+	+	+
7	—	—	—	+	+	+	+	+	+
8	—	—	—	+	+	+	+	+	+
2	+	+	+	-+-	-+-	—	—	—	+
3	+	+	+	-+-	-+-	—	—	—	+
4	+	+	+	-+-	-+-	—	—	—	+
5	+	+	+	—	—	—	—	—	+
6	+	+	+	—	—	—	—	—	+
P	+	+	+	+	+	+	+	+	—

Fig. 2. Results of reciprocal crosses among the 8 parthenogenetic diploid progeny and the parent (P)

Each plant was selfed and crossed with all the others and the parent reciprocally. The results of 8 plants all arising from the same parent, having the S constitution S_1S_2, are given in Fig. 2. Two plants (2 and 3) were temporarily pseudocompatible and six were self-incompatible. They fell into two intra-incompatible inter-compatible groups, one having three plants (1, 7 and 8) and the other five (2, 3, 4, 5 and 6), as would be expected if the plants comprised two S homozygous classes ($S_1S_2 \rightarrow S_1S_1 + S_2S_2$). The two pseudo-self-compatible plants were pseudo-cross-compatible with each other, and as males with plant 4 but not with plants 5 and 6, though all belonging to the same group. Pseudo-compatibility, here, refers to an unstable condition where pollinations during the early part of the flowering period were partly compatible, whereas the same pollinations done later, when plants were relatively exhausted, were incompatible. However, there was one feature of these plants which was completely unexpected: all plants were compatible with the parent as *males* as well as females, and this compatibility was complete and permanent.

In order to test these plants further, *S* homozygous plants were obtained through bud-self-pollination of the parent plant (Pandey, 1963). A family of 18 plants grown from such seed comprised 3 *S* genotypic groups containing 7 S_1S_2, 5 S_1S_1 and 6 S_2S_2 plants. All plants were self-incompatible and, with rare exceptions, intra-group crosses showed normal cross-incompatibility. Three plants of the S_1S_1 genotype and two of the S_2S_2 genotype, all perfectly normal in incompatibility behaviour, were reciprocally crossed with seven parthenogenetic diploid plants. It is apparent from the results, given in Figs. 3 and 4, that the breakdown in cross-incompatibility found between the parthenogenetic diploids and the parent also occurs, only slightly less intensively, between the parthenogenetic diploids and the bud-selfed progeny, and it is immaterial whether the parthenogenetic diploids are used as males or females in the crosses.

♀ PARTHENOGENETIC DIPLOIDS		♂ BUD-SELFED PROGENY S_1S_1			S_2S_2	
		2	12	13	7	8
S_1S_1	1	+	−	−	+	+
	7	+	−	−	+	+
	8	+	−	−	+	+
S_2S_2	3	+	+	+	+	−
	4	+	+	+	+	+
	5	+	+	+	+	+
	6	+	+	+	+	−

Fig. 3. Results of crosses between 7 parthenogenetic diploid progeny as females and 5 bud-selfed progeny, comprising 3 S_1S_1 and 2 S_2S_2 genotypes, as males

There is no doubt that the plants were of maternal origin. Since the pollinations were done in complete isolation, the possibility of contamination is ruled out. Maternal seeds could arise from rare diploid eggs, produced through abnormal meiosis, having the same *S* constitution as the mother, *i.e.* S_1S_2, or they could arise from normal haploid eggs. The present results suggest that the seeds, as stated earlier, arose through parthenogenetic diploidy, the haploid egg, stimulated without fertilization to double its chromosome number, producing a diploid embryo. Such maternal diploids are not uncommon in interspecific hybridization in solanaceous plants and have been amply investigated by other authors to show that they arise from parthenogenetic diploidy (Abdalla and Hermsen, 1972). This, in the present experiment, gave rise to two intra-incompatible intercompatible groups of plants having the *S* homozygous constitutions S_1S_1 and S_2S_2, with no plant having the maternal constitution S_1S_2. The individual plants, though perfectly homozygous, were still genetically different from each other, the egg having been a product of meiotic segregation. The genetic significance of these plants is that a sufficient number of entirely homozygous, genetically segregant plants have been produced from the same parent at one time, thus allowing a comparative examination of the efficiency of a specific genetic system under heterozygosity and complete homozygosity. The rare sporadic occurrence of this phenomenon in nature, usually in genetically undefined materials, has not allowed such an investigation previously.

♀ BUD-SELFED PROGENY		♂ PARTHENOGENETIC DIPLOIDS S_1S_1			S_2S_2			
		1	7	8	3	4	5	6
S_1S_1	2	+	+	+	+	+	+	+
	12	+	+	+	+	+	+	+
	13	+	+	+	+	+	+	+
S_2S_2	7	+	+	+	+	+	+	+
	8	+	+	+	−	−	+	−

Fig. 4. Results of crosses between 5 bud-selfed progeny, comprising 3 S_1S_1 and 2 S_2S_2 genotypes, as females and 7 parthenogenetic diploid progeny as males

The most remarkable feature of these plants with regard to incompatibility reaction is that they all behave abnormally in relationship with the parent plant. Pollen of both progeny genotypes S_1S_1 *and* S_2S_2 *are normally expected to be incompatible on the parental* S_1S_2 *styles* (♀ S_1S_2 × S_1S_1 ♂ *and* ♀ S_1S_2 × S_2S_2 ♂). *But, in fact, in this case they are all fully compatible. Reciprocal crosses of these plants with the identical S genotypes obtained through bud-self-pollination of the parent plant again show serious breakdown of cross-incompatibility. N. forgetiana* plants behave as perennials under our growing conditions. The original parent plant involved in this experiment, and a large number of its cuttings, have been grown for the last three years and show perfectly normal incompatibility reaction. The normal incompatibility in at least some of the combinations between bud-selfed progeny (♀) and parthenogenetic diploids (♂) (Fig. 4) rules out any possibility of pollen contamination in the original interspecific pollinations, or mutation of the *S* allele in the egg, being the cause of these unexpected results. What, then, is the basis of this major change in incompatibility behaviour?

3. Two-Tier System of Genetic Control

Self-incompatibility in *Nicotiana*, and a large number of other self-incompatible species, is under a two-tier system of control (Mather, 1943, 1949; Rendel, 1968): (1) A major locus *S* (gene complex) —

the structural gene, also called "switch" gene — which has a large number of highly specific alleles; and (2) numerous minor genes, also called modifiers or polygenes, some of which may be complementary and others additive in nature, which collectively comprise the polygenic component of the regulatory system. In homomorphic flowering plants, the physiological mechanism of incompatibility reaction is of the oppositional inhibitory type, as opposed to the complementary stimulant type occurring in some heteromorphic species (Lewis, 1954a). Thus in *Nicotiana*, and a large number of other homomorphic flowering plants, incompatibility is a positive reaction between pollen and style caused by an oppositional inhibition of the antigen-antibody type of reaction.

Normally, in self-incompatible species, plants are highly heterozygous, so that minor alleles are mostly in the heterozygous condition expressing the dominant phenotypes. It is considered that a heterozygous genetic background is essential for the proper functioning of the *S* alleles, for inbreeding often disturbs incompatibility. Inbreeding exposes the minor genes into homozygous recessive condition, and may therefore, in numerous ways, alter the balance of the polygenic component beyond the threshold necessary for the major switch gene to function properly (Mather, 1943; Pandey, 1959).

In the present experiment, pseudo-compatibilities in plants 2 and 3 expressed during the early flowering period may be due to dissociation of certain complementary genes through segregation. This presumably tends to disturb the threshold value of certain metabolic product(s) when the plants are in the early vigorous growing condition but not when the metabolic activity is at a lower ebb late in the flowering season. A similar strengthening of self-incompatibility because of poor nutrition physiology, poor growing conditions, or late stage of the flowering season, has been reported in usually inbred plants of several species (Adams, 1953; Eue, 1953; Pandey, 1956a, 1959, 1960; Myers, 1948). Other kinds of pseudo-compatibility normally found in inbred progeny, which are due to shifts in the required thresholds (Pandey, 1959), could also occur in these plants.

Parthenogenetic diploids of the kind reported here represent an extreme case of inbreeding where all genes are in the homozygous condition — a situation which, as indicated from Allard's work (1965, 1966), usually does not occur in nature (except in a polyploid condition).

The incompatibility reaction concerns the physiologies of two separate organs and involves two factors: (1) the corresponding specific reactants must occur both in the pollen and the style; and (2) the background physiological thresholds in the two organs must be in harmony so that the two specific reactants are in the *proper form and concentration* which allows the desired reaction to occur when brought together. The occurrence of generally normal self- and cross-incompatibility in the parthenogenetic diploid progeny suggests that the above two factors are not unduly disturbed through the complete loss of heterozygosity as long as the two participating organs are similar genetically. However, the situation is entirely changed when the style of a highly heterozygous parent is pollinated with the pollen of completely homozygous progeny. The breakdown of cross-incompatibility in these combinations is unequivocal and complete. What type of physiological difference between the heterozygous parent and the homozygous progeny could bring about such a consistent, dramatic change?

4. Integrated Heterozygosity

Since the genetic machinery controlling the incompatibility reaction, as shown by their incompatibility behaviour, is complete and functional, individually in both parent and parthenogenetic diploid progeny, the difference between the two, in the general sense, must be quantitative, not qualitative. That is, it is the second of the above two factors, balance between the thresholds of the two organs, which is causing this change. It would appear, therefore, that heterozygosity in the parent, and indeed in all normally heterozygous plants of a self-incompatible species, for the incompatibility reaction, bestows a physiological threshold of quite a different dimension from that which can be obtained in completely homozygous plants. The disparity in the threshold between the parent, which thus probably has a very wide range of incompatibility expression potential, and the progeny, which presumably have a comparatively limited potential, is such that the normally expected oppositional reaction is not possible, resulting in cross-compatibility. In a general sense, lack of incompatibility in the backcrosses can be attributed to the inherent lack of plasticity in the incompatibility system associated with the total loss of heterozygosity in the parthenogenetic diploids.

There are two phases in the physiology of the *S* gene action: 1. a primary *S*-gene action phase which occurs independently in the pollen and style, and 2. a secondary, incompatibility reaction phase which occurs after the pollen and style have come in contact with each other, and presumably involves conversion of incompatibility precursors into incompatibility substances (Pandey, in press). Observations from several independent sources suggest that the conversion of incompatibility precursors into incompatibility substances is dependent upon the relative polygenic backgrounds of the pollen and style (Pandey, 1962, 1968, 1969). It is likely that the disparity of the thresholds between pollen and style and the lack of conversion of incompatibility precursors into incompatibility substances are causally related phenomena.

As stated earlier, the physiology of incompatibility is governed by a two-tier system of genetic control, the major *S* gene and the regulatory polygenes specific to the *S* gene. The essential balance of thresholds between pollen and style is the function of the polygenes. The latter produces the physiological background in which the total expression of the *S* gene complex, including both phases, occurs, and is likely to be sporophytic in the pollen as well as the style. The fact that the action of the *S* gene itself in the pollen, by virtue of its gametophytic determination, is naturally selected against the adverse effects of complete homozygosity is irrelevant here since the cytoplasmic background would already have been determined beforehand sporophytically.

The consummation of the incompatibility reaction comprises a number of component processes of which only a part, particularly that which governs the production of specific incompatibility precursors, is controlled by the *S* gene; when, how much, or whether these incompatibility precursors from the pollen and style are actually converted into incompatibility substances, and interact to produce the incompatibility reaction, is very much under the polygenic control. It is proposed here that the function of the INTEGRATED heterozygosity located in the polygenic component is to ensure the genetic background necessary for a wide efficiency of the *S* gene system in producing incompatibility. Parthenogenetic diploidy destroys the INTEGRATED heterozygosity, thereby eliminating the buffering system built into the physiology of incompatibility mechanism.

5. Heterozygosity as an Integral Factor in Genetic Systems

Heterozygosity is the most important consequence of the evolution of meiosis, diploidy and sex, and constitutes one of the key factors in the evolution of complex organisms (eukaryotes). Mechanisms to achieve it are developed in one form or another, and to varying degrees, in all principal lines of evolution in plants (Pandey, 1969), with self-sterility being the most efficient. In angiosperms, there is ample evidence to suggest that self-incompatibility occurred very early in their evolution, the present self-compatible species having been derived from the originally self-incompatible ancestors (Whitehouse, 1950; Stebbins, 1950, 1957; Lewis, 1954a, b; Lewis and Crowe, 1958; Pandey, 1956b, 1959, 1960). In animals, owing to the general occurrence of unisexuality, heterozygosity is even more consistent throughout their evolution. Heterozygosity is thus an ancient constant feature and stimulant of evolution. Its integration with genetic systems controlling certain basic features in evolutionary and developmental mechanisms is therefore not unexpected (Pandey, 1972). Genetic mechanisms have probably evolved to keep the destruction of the INTEGRATED heterozygosity to the minimum. Parthenogenetic diploidy, however, is the most effective method of destroying this heterozygosity.

A normally cross-pollinated species with high heterozygosity when forced to inbreed may face genetic crisis at several levels. The most commonly understood of these is the expression of recessive lethal, sub-lethal or deleterious genes, which in the heterozygous condition are usually masked by the normal dominant genes. It is suggested here that this may be only the most apparent consequence of forced homozygosity in a normally outbreeding species. The physiological consequences, to development and plasticity, of dislocation in the integrated genetic system attendant with the abolition of heterozygosity altogether may be far more significant than is realized at present.

If the genetic system of species includes a number of major genes or gene complexes controlling physiologically integrated pathways, and each of these has its associated set of minor genes forming a polygenic component of the regulatory system, as exemplified by the incompatibility mechanism, it is possible that preservation of certain kinds of heterozygosity in each of the constituent minor gene systems may be an important element in the buffering efficiency and, hence, total continuity of the genetic system and its evolutionary potential. Such heterozygosity, it is suggested, may have become integrated, during evolution in the past, with the innumerable genetic systems comprising the total physiology of the species. From this point of view, the descendant present species as a whole may not normally be affected to a great degree by the prevailing breeding system, whether outbreeding or inbreeding: Rigorous natural selection would maintain the INTEGRATED heterozygosity if the network of physiological systems concerned was still operative. Furthermore, conservatism of developmental patterns of interconnected adaptive pathways may be such that many units of INTEGRATED heterozygosity will persist after the selective pressure that brought them into being no longer exists. The heterozygosity concerning the present hypothesis has a much deeper base than that envisaged in Lerner's hypothesis (1954) of "developmental homoeostasis", the effects of which were believed to be limited to cross-fertilized organisms; or that envisaged in its modification, proposed by Griffing and Langridge (1963), which affects both self- as well as cross-fertilized organisms, but which is still dependent on SEGREGATIONAL heterozygosity.

DNA studies by *in vitro* hybridization techniques have shown that somewhat less than 50 per cent of the DNA of mammals studied occurs in the so-called repetitive sequences (Britten and Davidson, 1969). It has also been shown that parts of the genome are translated but never appear as cytoplasmic RNAs or structural proteins. Wallace (1963), Stebbins (1969) and Britten and Davidson (1969) have pointed out that much of the difference between higher species and faunal groups may reside in the dissimilarities of regulating mechanisms (Richmond, 1970). Thus a considerable proportion of the polygenic components of specific genetic systems may belong to regulating mechanisms, in which allelic variations with their additive, codominant, competitive or complementary effects, would be integrated with the specific major genes of the systems. It is conceivable that heterozygosity of certain genes in the polygenic component, some of which produce and others do not produce cytoplasmic RNAs or structural proteins, might be an essential element in the evolution of such integrated systems. Darlington and Mather (1952) and Lewis (1954c) have classified allelic action and interaction in five types: direct, successive, co-operative, competitive and parallel. In the last, parallel gene action, a particular phenotype is the result of two or more alleles working towards the same end but by slightly different synthetic routes. Fincham (1972) has discussed the different ways in which heterozygous advantage can be realized on the molecular level, and argues strongly in favour of the view that heterozygous advantage is the general basis for enzyme polymorphisms so commonly observed in nature.

INTEGRATED heterozygosity has certain attributes in common with B chromosomes (Rees and Jones, 1972), in that (1) both may not be indispensable to specific physiological systems as such but only to organisms as a whole, or to specific populations and ecotypes, and (2) both, primarily, have quantitative rather than qualitative effects, and seemingly determine the plasticity and adaptability of the organism. INTEGRATED heterozygosity and B chromosomes may include elements which "repair" or "boost" the expression of the essential segments of the genome, major genes, or gene complexes, which might otherwise become deficient or degenerate owing to conflicting selection pressures on interlocking metabolic pathways (Pandey, 1969).

Normally, the requirement of heterozygosity in genetic systems may be considerably buffered by the presence of duplicate, but slightly redifferentiated, genes dispersed in the genome. Thus, theoretically homozygous organisms may have a large amount of "heterozygosity" of this nature hidden in their genome. The apparent normalcy, or near normalcy, of individuals which are highly or entirely homozygous may be largely due to the occurrence of such "heterozygosity" locked in the widespread duplications in the genome. In this context, the phenomenon of heterosis, with its attendant wide plasticity, may be considered as the other extreme where most requirements of heterozygosity are satisfied abundantly. Darlington (1958), Darlington and Mather (1952), Dobzhansky (1966), Simpson (1964), and others have pointed out that natural selection does not act on separate genes or traits but on whole genotypes and phenotypes. Selection for single genes is believed to be a rare phenomenon. In the light of the present discussion selection must be seen as acting on genetic systems rather than on individual genes.

If the present hypothesis is true, that there is a certain degree of INTEGRATED heterozygosity in the minor genes comprising polygenic regulatory components of major genes and complexes, then the *preservation* of this heterozygosity may be an integral part of the genetic systems of many, probably all, eukaryotic organisms in nature. *Widespread heterozygote advantage of single genes as observed through isozyme studies may thus have a deeper significance in development, adaptation, and evolution.*

It may be appropriate here to recall Darlington (1958): "Just as the Mendelian principle of segregation enables us to predict the results of simple breeding experiments so the Darwinian principle of natural selection enables us to predict the results of simple selection experiments. But in the changing states of natural populations with their alternations of stagnation and crisis, their cycles of stability and breakdown, *the initiative often passes to internal conditions, to the genetic system. Inbreeding then does not always purify. Selection, external selection, does not always direct.*" (italics added.)

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