AN EVALUATION OF THE MULTIPLE CROSSOVER AND SIDE-CHAIN HYPOTHESES BASED ON AN ANALYSIS OF ALPHA DERIVATIVES FROM THE A^b-P COMPLEXES IN MAIZE[1]

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VARIOUS gene complexes which behave as alleles of A_1 in maize, and which carry the general designation A^b, give rise infrequently, but consistently, to a characteristic mutant form, designated A^a or alpha (α), with intermediate effects on plant and aleurone phenotype. The occurrence of this mutant form is associated with one of the two types of recombinants for spanning marker loci with a far higher frequency than anticipated on the basis of coincidental exchange in the marked segment (LAUGHNAN 1949, 1952a, 1955a). It was concluded that alpha is itself one element of a linked complex that also includes a member, designated beta (β), whose phenotypic effect on plant and aleurone is full purple and is, on most criteria, indistinguishable from the total effect of the A^b complex itself. The isolation (LAUGHNAN 1956, 1961) of the beta element from the various A^b complexes in strands whose recombinant markers are complementary to those carrying the alpha isolate confirms both the interpretation of alpha and beta as closely linked but separable elements, and the assignment of sequences to the members.

However, as it stands, the scheme described above does not provide an obvious explanation for the infrequent but regular occurrence, from these same A^b complexes, of alpha-carrying strands that are nonrecombinants for the marker loci (LAUGHNAN 1949, 1952a, 1955a, c, 1961). The purpose of this report is to consider, for their bearing on the origin of the nonrecombinant alpha cases, certain specific mechanisms which have been proposed to account for the occurrence of somewhat similar exceptions in Neurospora (GILES 1951; MITCHELL 1955; ST. LAWRENCE 1956; FREESE 1957; CASE and GILES 1958; STADLER 1959), in Aspergillus (PRITCHARD 1955, 1960a, b; CALEF 1957), in yeast (ROMAN and JACOB 1958), in bacteriophage (STREISINGER and FRANKLIN 1956; CHASE and DOERMANN 1958), in maize (STADLER and NUFFER 1953; EMMERLING 1958), and in Drosophila (DEMEREC 1926, 1928; CHOVNICK 1958).

In most of these studies single-strand analysis of wild-type recombinants from heterozygotes involving two closely linked, functionally related mutants gave consistently anomalous distributions for the outside marker combinations. Data (Table 1) on meiotic recombination between two cysteineless mutants (STADLER 1959) in Neurospora are typical of inconsistencies encountered in similar in-

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TABLE 1

Distribution of marker combinations among cysteine-independent spores from the cys-c/cys-t *heterozygote**

Cross	Selection		Strand types with respect to marker loci: Recombinants *un ylo*	Recombinants + +	Nonrecombinants *un* +	Nonrecombinants + *ylo*
un cys-c + ** / + *cys-t ylo*	Cysteine independent	No.	18	25	68	26
		Percent	13.1	18.3	49.6	19.0

* This table is adapted from STADLER 1959.
** Map distances established by conventional crossover analysis of progeny from this cross: *un* 2.1 *cys* 5.6 *ylo*.

vestigations of other loci. If, for example, it is held that the selected cysteine independent arises by some mechanism not involving the participation of both cysteineless cistrons, an argument which is at odds with the observation that self (within strain or homoallelic) crosses yield prototrophs with extreme rarity, or not at all, we find that among prototrophs the frequency (31.4 percent) of strands that are recombinant for the outside markers is far in excess of the predicted value (7.7 percent) for coincidental exchange based on the genetic map length of the *un–ylo* segment. On the other hand, the argument that the *cys* independent results from conventional exchange between homologues within the *cys* cistron, which involves the assumption that *cys-c* and *cys-t* occupy different sites, is at variance with the high frequency of strands that are nonrecombinant for the outside markers, and with the near equality of the two recombinant classes. In this and in other studies yielding similar contradictory results, it is evident that the event giving rise to nonmutant progeny has neither the characteristics of gene mutation nor of conventional crossing over but might be considered to have attributes of both.

The various schemes proposed to explain these unusual results have, in addition, had to take account of the unexpected occurrence of aberrant asci in tetrad analyses of individuals heterozygous for closely linked loci. Thus, again using the studies on the cysteine mutants (STADLER 1959) as an example, tetrad analysis of the *cys-c/cys-t* trans heterozygote gave three asci, of the 153 analyzed, that carried a cysteine independent spore pair. Backcross tests of the nine cysteineless strains from these asci established that the *cys-c cys-t* double mutant, expected if the recombinational event giving rise to the wild type were reciprocal, was not represented. Similar anomalous results, featuring the absence of the complementary recombinant type, have been obtained at other loci in Neurospora (MITCHELL 1955; CASE and GILES 1958), in yeast (LEUPOLD 1958; ROMAN and JACOB 1958), and in Aspergillus (STRICKLAND 1958). It should be emphasized, however, that recombinations yielding the wild type are not in all cases nonreciprocal; tetrad analyses involving pantothenic acid mutants (CASE and GILES 1958) reveal that reciprocal recombination also occurs, and in Aspergillus, where STRICKLAND (1958) analyzed a number of loci for this phenomenon, only rare instances of nonreciprocal recombination were encountered.

The hypotheses

Two basic schemes have been proposed to account for the occurrence, as a nonreciprocal recombinant, of the wild-type revertant from the type m_1/m_2 heterozygote, and for the unexpected distributions of outside markers among such recombinants. These models are treated here in some detail so that we may consider, in the sections that follow, whether they represent valid explanations for the anomalous alpha derivatives in maize.

Single mechanism: According to this scheme both reversion to independence, and recombination for outside markers, are ascribable to the same basic phenomenon. On the basis of extensive analyses of mitotic and meiotic recombinants within the *ad8* cistron in Aspergillus, PRITCHARD (1955, 1960a,b) has proposed that recombination takes place in localized regions of effective pairing. The evidence he presents for a linear separation of the various mutant forms in this cistron is convincing. Assuming that the adenine independents which are selected in his experiments result from crossing over between mutational sites in various trans heterozygotes (e.g. *ad8/ad11*) the excessive recombination for outside markers among these revertants (negative interference) is explained on the basis of multiple exchanges within a short, effectively paired region which spans the *ad* cistron. On the hypothesis of localized, effective pairing, the apparent high negative chromosome interference observed among the selected revertants does not imply that one crossover enhances the probability of another in its neighborhood but rather follows logically from the assumption that (a) effective pairing in the vicinity of the adenine locus occurs in only a small fraction of the cell population sampled, and (b) when pairing is realized, the probability of exchange within the paired region is sufficiently high (PRITCHARD estimates 0.6) to account easily for the occurrence of multiple crossover strands.

On a copy-choice model the author (PRITCHARD 1960a) proposes that duplication of the two new strands progresses simultaneously and that switches in copy from one template to the other, which may occur only within the paired segment, are highly correlated in Aspergillus, thus accounting for the regular occurrence there of reciprocal recombinants. PRITCHARD's suggestion that nonreciprocal recombination, as encountered notably in Neurospora and yeast, may be attributed to slight displacement of the point of switching of one duplicating strand with respect to the other, is not unlike the explanation offered by FREESE (1957) who also postulates regions of localized, intimate pairing within which multiple switches may occur at the time of duplication; but on the latter scheme the two duplicating strands need not have equal numbers of switch points within the pairing region.

Since maize does not offer the opportunity of tetrad analysis, it is not possible to say whether the exceptional alpha occurrences are reciprocal, nonreciprocal or both. The essential feature of the schemes based on a single mechanism, so far as they apply to the case in maize, is that multiple exchanges within relatively short (PRITCHARD estimates a mean length of pairing region of 0.4 of a conventional map unit), effectively paired segments, whether by copy-choice or by exchanges between previously duplicated strands, are responsible for both the

reversion event and the excessive recombination for outside markers among the selected revertants. Specifically, on this hypothesis, the alpha isolations from A^b compounds in maize are all attributable to crossing over; accordingly, those that are nonrecombinants for markers must represent isolations on strands that have been involved in an even number of exchanges in the marked segment.

Separate mechanisms: The various schemes that fall in this category assume that reversion at the locus under investigation, and the excessive recombination for outside markers noted among the selected revertants, are due to different mechanisms. However, since there is a high correlation between reversion and recombination for outside markers, it must be assumed that both events are favored by a third condition which is satisfied in some, but not all, individuals of the population under investigation.

Perhaps the most appealing basis on which to argue separate events is to consign them to different spatial realms. Thus, ROMAN and JACOB (1958) favor the concept of a branched chromosome to explain the correlation between reversion at the isoleucine locus and recombination between the histidine-1 and tryptophane-2 marker loci in yeast, and in particular to account for the differential effect of ultraviolet radiation on these two processes. According to this model, in the trans heterozygote involving two isoleucine mutants designated i_a and i_b, the latter are considered to represent defects at different sites in a DNA side chain (cistron). The selected isoleucine-independent revertant results from recombination between the differently-defective side chains to produce one that is functionally normal, though it is clear that this event itself must be nonreciprocal to explain the observation that the complementary double-mutant type does not occur simultaneously. The abnormally high rate of recombination for outside markers among the revertants is explained by assuming that both the hypothetical side-chain event and conventional crossing over in the protein backbone are favored when some third condition, such as intimate pairing of homologues in the isoleucine region, obtains. On this scheme, ultraviolet radiation is held to affect differentially the side-chain and backbone events, though the authors agree that the results are not inconsistent with a scheme, such as that proposed by FREESE (1957), involving a single mechanism without the assumption of side chains.

In testing the fit of the maize results to predictions based on this hypothesis it should be emphasized that this scheme does not provide for a linear separation of closely linked, mutant sites along the backbone of the chromosome and hence prohibits the occurrence of a revertant as a result of a conventional crossover. Therefore, neither of the recombinant marker classes should be favored on this account though they may still be disproportionate if the two kinds of side-chain reversions have different frequencies and if the frequencies of coincidental crossovers in the marked segments to left and right are different.

Because it may have special significance for the anomalous derivatives in maize, a variation of the conservative side-chain hypothesis presented above should be considered. According to this modification the closely linked, mutant sites reside in separate but adjacent side chains. STADLER (1959) has proposed

and favors such a model to explain the results obtained from the analysis of the cysteineless mutants; thus, in the case of the *cys-c/cys-t* heterozygote, one homologue carries an impaired *cys-c* side chain and an adjacent side chain that is normal for the *cys-t* impairment, while the other homologue carries the complementary condition. Although this model suffers the disadvantage of spatial discontinuity within the cistron, it allows for the occurrence of reversion either as a result of a crossover in the backbone or as a result of side-chain events. According to this scheme the alpha and beta members of A^b complexes in maize are considered to reside in different but adjacent side chains.

In her original report on the pyridoxine mutants, MITCHELL (1955) proposed that the occurrence, in asci from trans *pdx/pdxp* heterozyogtes, of occasional pyridoxine independent spore pairs unaccompanied by the reciprocal double-mutant type might be attributed to gene conversion (WINKLER 1930) leading to the recovery, in a single duplicating strand, of the normal counterparts of both mutant forms, for which she did not assume linear separation. Since gene conversion itself will not account for the excessive recombination of outside markers encountered among the revertants in single strand analyses, it was assumed that both gene conversion and crossing over are favored by a particular circumstance, held to prevail in some cells of the population but not in others, such as intimate pairing between homologues in the neighborhood of the pyridoxine locus. Unfortunately, the term "gene conversion" has come into widespread use to identify, in a general way, those recombinational phenomena which, though they are correlated with exchanges between outside markers, can not be the product of conventional crossing over alone. But considered from an operational standpoint, "gene conversion" has usually been defined loosely or not at all, and has come into use to describe results, in much the same way that "position effect" may be used to describe a phenomenon, but not to explain it. In the writer's opinion, if the term "gene conversion" has been loosely defined, or misapplied, the fault lies mainly in the lack of a convincing conceptual basis for the phenomenon. Gene conversion is held to mean (WINKLER 1930) that alleles in heterozygotes are able to convert *inter se* their counterparts in homologues, thus to explain the occasional exceptional ratios encountered in asci (LINDEGREN 1949, 1953, 1955). But the most reasonable basis for the occurrence of such an event is by partial or complete substitution of the genetic material of one allele by the other occurring as a result either of an aberrant duplication or of exchanges, which are not exactly reciprocal, between already duplicated strands. Thus, if it were established beyond doubt that multiple exchanges within short segments of a chromosome actually do occur, and that they may on occasion be nonreciprocal, the presumed cases of conversion would be credited to this phenomenon if only because a mechanism for gene conversion, as precise as that for multiple exchange, has not been formulated. It is not surprising, therefore, that those who have used the term "gene conversion" to describe superficially the results they encounter have in many cases proposed one or another more specific recombinational mechanism to explain them. In any case, as will be evident later, in the interpretation of the maize experiments it is impossible to distinguish operationally between gene

conversion and the more clearly defined recombinational mechanisms discussed above and for obvious reasons the writer prefers to deal with the latter types of hypothetical models.

The A^b *complexes in maize*

Three A^b complexes of separate origin have been the objects of intensive genetic analyses. One of these, here designated A^b-Ec, is the original A^b of Ecuador extraction whose unusual phenotypic effects were described by EMERSON and ANDERSON (1932). The other two, having the general designation A^b-P, and referred to specifically as A^b-Lima and A^b-Cusco, were originally isolated from Peruvian accessions; they have a similar basic structure though they exhibit slight differences in action which indicate that they are not identical.

It has been established (LAUGHNAN 1952a, 1955a) that both the A^b-Ec and A^b-P forms are complexes consisting of separable alpha and beta elements, but it is also evident, from previous investigations, that these complexes exhibit rather remarkable differences which may be summarized as follows:

(a) The alpha element of the A^b-P complexes is decidedly weaker in its effect on plant and aleurone pigmentation than that of A^b-Ec.

(b) Crossover analysis reveals that the sequence of alpha and beta elements in the A^b-P complexes is the reverse of that in A^b-Ec; thus, using the symbol C to designate the centromere, the order of members in the A^b-Ec complex is C:α:β whereas the sequence of members in the A^b-P complexes is C:β:α.

(c) Whereas the A^b-Ec and A^b-P complexes yield alpha derivatives that are recombinants for marker loci with similar frequencies (*ca.* 3×10^{-4}), there is a tenfold difference in the frequency of occurrence of the non-recombinant alpha derivatives from the A^b-Ec complex (*ca.* 5×10^{-5}) as compared with that from the A^b-P complexes (*ca.* 5×10^{-4}).

Analyses (LAUGHNAN 1952b, 1955b) of the derivatives from homozygous A^b-Ec individuals, and from certain heterozygotes involving A^b-Ec, indicate that alpha and beta, or the segments in which they reside, are members of an adjacent duplication in which the genetic materials are ordered in the same sequence; it is evident that A^b-Ec, like Bar in *Drosophila melanogaster*, is a tandem, serial duplication whose members engage in oblique synapsis with homologous elements of the homologue. Moreover, similar evidence (LAUGHNAN 1955a, 1961) from A^b-P homozygotes establishes that the beta- and alpha-carrying segments of this complex engage in oblique synapsis, indicating that they too are members of a tandem, serial duplication. Finally, the occurrence of viable alpha derivatives from A^b-Ec/A^b-P heterozygotes (LAUGHNAN 1955a) establishes that these complexes are not simply gross inversions with respect to each other, but rather that they have their genetic material ordered in the same sequence but with the alpha and beta segments interchanged as represented below (the numbers here have the significance only of indicating sequence of genetic material):

A^b-Ec						A^b-P					
α			β			β			α		
1	2	3	1	2	3	1	2	3	1	2	3

On the basis of these models for the two complexes there is no difficulty in accounting for the occurrence of the recombinant alpha (pale) derivative; since alpha is the left-most element of the A^b-Ec complex it is isolable on a strand that is recombinant for the distal marker locus, and conversely, crossover isolation of alpha from the A^b-P complex is associated with recombination for the proximal marker. The pale phenotype of both crossover isolates is thus assignable to loss, to the complementary crossover strand, of the purple-acting beta element which masks the alpha effect when they are associated in the complex.

But the isolation of alpha (or loss of beta effect) from these complexes as a nonrecombinant for the marker loci does not follow from simple inspection of the model presented above and therefore requires special consideration. One of the mechanisms proposed to account for the occurrence of the nonrecombinant alpha derivatives is gene mutation of the beta member to a null level. Such a qualitative change in the beta member, rendering it impotent in the production of pigment, would lead directly to the expression of the adjacent alpha member; and since, on this hypothesis, the homologue does not participate in the mutational event, the alpha occurrence, except for randomly distributed, coincidental exchanges, would not be associated with recombination for marker loci.

This possibility has been explored experimentally using three separate approaches:

(a) Beta elements, isolated by crossing over from the alpha member of A^b-P complexes, were subjected to mutational analysis in heterozygotes with the complex from which they were derived, to analyze for the hypothetical beta mutational event (LAUGHNAN 1957, 1961).

(b) The frequency of occurrence of the nonrecombinant alpha was investigated in a synthetic complex of α:A constitution in which the A member was substituted for beta in the α:β complex of A^b-Ec (LAUGHNAN 1961).

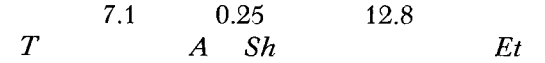
(c) Nonrecombinant alpha derivatives, along with corresponding crossover isolates from both complexes, were themselves subjected to crossover analyses in attempts to isolate the hypothetical null form of beta which is presumed, on the gene mutation hypothesis, to be present in the alpha of nonrecombinant origin (SARMA 1959).

These investigations indicate (LAUGHNAN 1955c, 1961) that mutation of the beta member of the A^b complexes to a null form, if it occurs at all, is so infrequent that it can not be the mechanism responsible for the occurrence of the vast majority of nonrecombinant alpha derivatives.

In considering the possibility that the models discussed in a foregoing section might account for the origin of the anomalous alpha derivatives in maize, it should be emphasized that the typical system employed in the pertinent studies with microorganisms involves a trans type heterozygote in which each of the homologues carries a different mutant or defective site within a specified functional unit or cistron. Characteristically, each of these mutant strains and their heterozygote are unable to grow in the absence of a specific metabolite, whose synthesis proceeds normally in the presence of an unimpaired cistron. But among the progeny of the heterozygote produced in mitotic or meiotic cycles, as the

case may be, there are rare occurrences of revertants that no longer require the metabolite. These revertants are the highly selected sample of the population which is the object of further genetic analyses. Since the individual mutant strains themselves show only negligible rates of reversion, the selection of revertants from the heterozygote appears to enforce the participation of both homologues in the reversion event. On the other hand, the A^b complexes in maize carry the alpha and beta sites in a single homologue in what, by analogy, might be considered the cis phase; in pursuing the analysis of derivatives from such a complex, attention is focused on the isolation of the alpha member, an event which, unlike the reversion dealt with in the microorganisms, need not necessarily involve the homologue.

The figure below provides a map of a portion of the long arm of chromosome 3 in maize, including the *A* locus and the various marker loci employed in the



experiments to be discussed. The symbol *T* refers to translocation 2-3d (ANDERSON and BRINK 1940) which has been used extensively to mark a segment proximal to the *A* locus; it refers specifically to a 2^3 chromosome carrying the *A* locus in the interchanged portion of chromosome 3. In translocation heterozygotes, the symbol *N* will refer to a noninterchanged chromosome 3. Since marked heterozygotes to be analyzed for alpha occurrences were routinely crossed with homozygous testers not carrying the translocation, the presence or absence of the interchanged chromosome in exceptional offspring was easily determined by the presence or absence of 50 percent aborted pollen, the former of which is typically associated with plants that are heterozygous for the translocation; and since ears of plants heterozygous for this translocation regularly exhibit abortion of half of their ovules, the pollen classification referred to above was routinely confirmed by scoring the ears of exceptional individuals for the aborted condition. The sh_2 (shrunken-2) factor, which in homozygous recessive condition effects a drastic collapse of the mature endosperm, is distal to *A* and marks a segment whose genetic length is only one-quarter unit. Because of its close proximity to *A*, sh_2 has been preferred as a marker to the recessive factor *et* (etched endosperm, virescent seedling), but as we shall see, the latter may be used to advantage in certain instances. It should be observed that the genetic lengths of the *T* and *sh* marked segments on either side of the *A* locus, 7.1 and 0.25 units, respectively, are almost exactly the same as the corresponding segments, 6.0 and 0.2, on either side of the *ad8* cistron, defined by the markers *bi* (biotinless) and *y* (yellow), in PRITCHARD's (1960b) studies with Aspergillus.

In considering the results of investigations of the A^b complexes for their fit to the models discussed in a foregoing section we shall, for the sake of convenience, use the term *multiple exchange* in referring to the scheme that attributes both the reversion (primary event) and the excessive recombination for outside markers (secondary event) to a single mechanism, that is, to crossing over. The

alternative model, according to which the primary and secondary events are based on separate mechanisms, will be referred to as the *side-chain* hypothesis. In pursuing the analysis we may anticipate obtaining the answers to two different but not unrelated questions: (a) is it possible to explain the occurrence of both the recombinant and nonrecombinant alpha isolates according to one, both, or neither of these two hypotheses, and (b) if neither scheme represents a plausible explanation for the alpha occurrences, to what extent do the available data bear on whether these mechanisms may nevertheless operate in maize?

Evidence from deficiency heterozygotes

According to the multiple-exchange hypothesis, the alpha component of the marked heterozygote carrying the beta:alpha complex in one homologue and recessive *a* (associated with colorless phenotype) in the other, may be isolated as an apparent nonrecombinant for the marker loci in one of two ways, both of which involve double crossover strands (Figures 1B, 1C). One of these carries the parental markers of the A^b-P chromosome while the reciprocal event leads to an alpha strand carrying the parental markers of the homologue. But in either case one of the exchanges, hereafter referred to as the primary event, must occur within the complex, that is, between the beta and alpha members. If both the recombinant and nonrecombinant alpha derivatives are dependent on a crossover between the beta and alpha members, it would be anticipated that A^b-P/Df *a*-X hemizygous individuals, in which the homologue is deficient for a segment of the chromosome including the *A* locus and in which, therefore, the opportunity for synapsis of the beta:alpha complex and hence for exchanges between homologues in that region is removed, would yield no alpha offspring.

The data presented in Table 2 are pertinent to this argument; they concern both A^b-Lima and A^b-Cusco in hemizygotes involving *a*-X1 and *a*-X3, which are of X-ray origin (STADLER and ROMAN 1948) and are known to be deficient for a

TABLE 2

Alpha derivatives from hemizygous A^b-*P/Df* a-*X* individuals*

Source	A^b-P gametes tested	Distribution of alpha strands among offspring
T A^b-Lima *Sh/N a*-X1	161,240	109 *T α Sh*
T A^b-Cusco *Sh/N a*-X1	400	1 *T α Sh*
T A^b-Lima *Sh/N a*-X3	45,480	29 *T α Sh*
T A^b-Cusco *sh/N a*-X3	2,350	3 *T α sh*
No proximal marker:		
N A^b-Lima *Sh/N a*-X1	128,280	83 *N α Sh*
N A^b-Cusco *Sh/N a*-X1	86,355	44 *N α Sh*
N A^b-Lima *Sh/N a*-X3	4,555	4 *N α Sh*
N A^b-Cusco *Sh/N a*-X3	2,500	1 *N α Sh*
Totals	431,160	274

* The deficient segments of both Df *a*-X1 and Df *a*-X3 include the *A* locus and the *Sh* locus, as explained in the text.

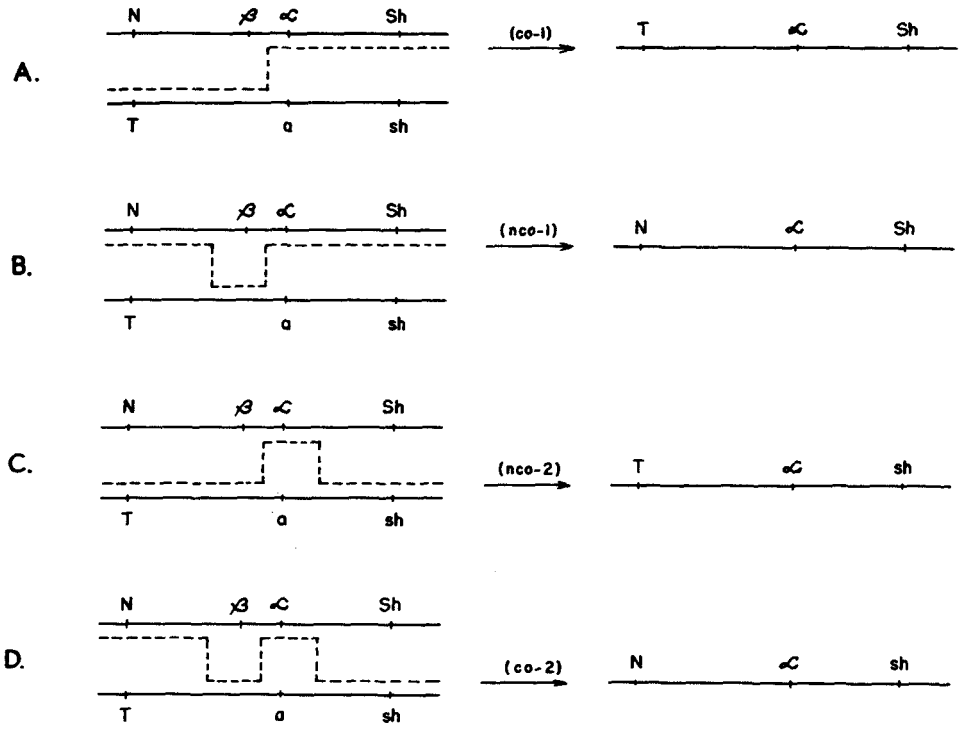


FIGURE 1.—Theoretical expectations for types of alpha-carrying strands from A^b-P/*a* heterozygotes on the multiple-exchange hypothesis. According to this scheme, all alpha isolations are attributable to the primary event, a crossover between beta and alpha members, held to occur when a relatively short segment involving the *A* region is effectively paired. The various combinations for marker loci among the alpha strands are functions of the number and position of additional exchanges (secondary events) within the effectively paired region. Complementary strands are not shown but, since the studies reported here deal with single strand analysis, it makes no difference whether the events diagrammed above result from switches on a copy-choice model or from exchanges between previously duplicated strands. The systematic basis for nco and co designation of alpha-carrying strands with regard to marker combinations is explained in a footnote to Table 3.

segment including the *A* locus and extending to the right beyond the *sh* locus. Contrary to expectation, these hemizygotes yield alpha progeny. Since these alpha derivatives arise from plants heterozygous for a deficiency in which there was no opportunity for exchange within the A^b complex, it may be concluded that they could not have occurred as a result of multiple exchange. Data to be presented elsewhere indicate that nonrecombinant alpha cases are at least as frequent among the progeny of these deficiency heterozygotes as they are among the offspring of sib A^b-P/*a* heterozygotes. Thus, while the results presented in Table 2 can not be taken to indicate that multiple exchanges, as postulated, do not occur in maize, they clearly indicate that some other mechanism must be responsible for the occurrence of the vast majority of nonrecombinant alpha derivatives.

We shall come later to the independent question of whether the multiple exchange hypothesis is valid for maize.

Although both *a*-X1 and *a*-X3 occurred among the progeny of X-ray treated pollen carrying *A*, and the genetic evidence indicating that they are deficiencies is decisive, it has not been possible to identify them cytologically. Moreover, there is no known proximal marker sufficiently close to *A* to define accurately the left-most limits of these deletions. We have therefore made appropriate crosses to analyze for alpha occurrences among the progeny of a special type of hypoploid in which one of the chromosome 3 homologues is deficient for almost all of the long arm of chromosome 3 and the other is involved in the translocation 2-3d interchange. Owing to nondisjunction in the second division of the microspore, hypoploid individuals occur regularly among the progeny of pollen parents carrying reciprocal translocations involving the A and B type chromosomes (ROMAN 1947). The hypoploid of interest here (Figure 2) was produced by crossing homozygous *T* A^b-Lima *Sh* egg parents with pollen parents carrying the A-B translocation known as T-B3a, in which almost all of the long arm of chromosme 3 is translocated to the centric portion of the B chromosome. These plants are easily distinguished from their genetically balanced sibs since they are poor in vigor, are about one third to one half normal height, and exhibit characteristic morphological deviations from normal. Moreover, they show the high frequency of abortion (70–80 percent) of both pollen and ovules predicted from their unusual chromosome constitution. It should be emphasized that the $T\beta$:αSh segment of these hypoploids (see Figure 2) has no homologous segment with which to pair and that the 2^3 chromosome, of which it is a part, is expected to be involved exclusively in synapsis with the chromosome 2 homologue.

Yet, among the relatively small population of 6,900 tested A^b-Lima gametes from backcrosses of these hypoploids with homozygous *N a sh* pollen, there were five alpha occurrences. Further analysis has confirmed that these cases are legitimate and that all are of the expected nonrecombinant type: *T* α *Sh*. Although the cases are too few to provide a reliable estimate of the frequency of the event, the rate as it stands compares favorably with that of alpha cases from the *a*-X hemizygotes (Table 2). The occurrence of alpha derivatives from this hypoploid, in which crossovers in the *T* β:α *Sh* segment are not possible, is critical evidence

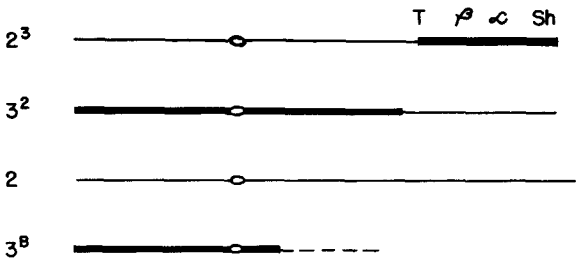


FIGURE 2.—Diagrammatic representation of the hypoploid individual in which one of the chromosome 3 homologues is deficient for most of the long arm of chromosome 3. See text for details.

indicating that the primary crossover event assumed on the multiple-exchange hypothesis is not required for nonrecombinant alpha isolation.

These results have a bearing also on the validity of the side-chain mechanism for the origin of nonrecombinant alpha cases. The more conservative version of this hypothesis, according to which the beta- and alpha-carrying segments are adjacent members in a single side chain, is illustrated in Figure 3. According to this scheme the primary event, leading to alpha isolation, is an exchange (or a switch on the copy-choice model) involving the side chains of homologous chromosomes, whereas the secondary events, tending toward the randomizing of marker combinations, are considered to be crossovers occurring coincidentally between the backbones of homologues. We see from Figure 3 that regardless of the sequence assumed for alpha and beta in the side chain, the corresponding side chain of the homologue must be involved in the alpha isolation. From the data presented above having to do with alpha occurrences from the A^b-P/*a*-X hemizygotes (Table 2) and from the T-B hypoploid, neither of which provides the required homologous side chain, it is obvious that the conservative side-chain hypothesis can not be the basis for the occurrence of the great majority of alpha cases.

The modified version of the side-chain hypothesis would have the alpha and

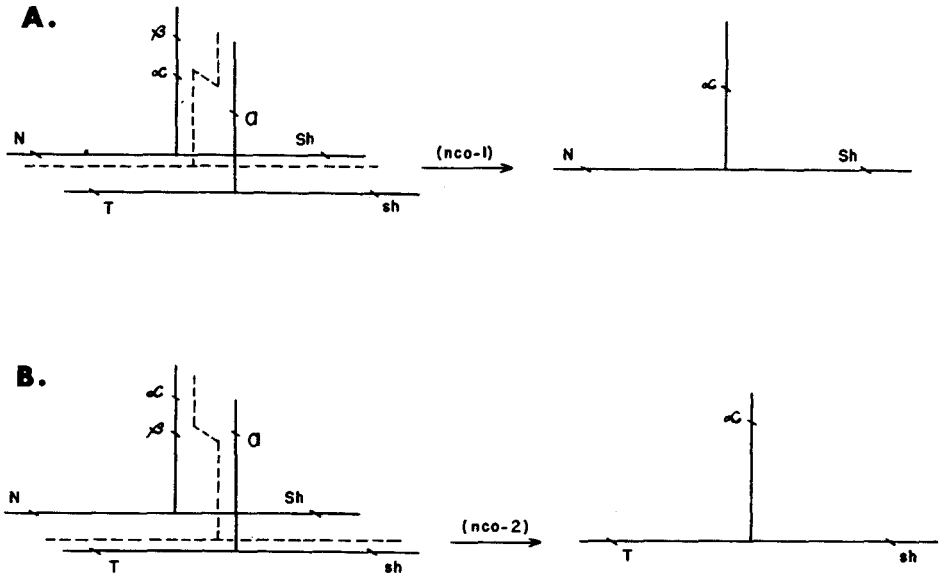


FIGURE 3.—Diagrammatic representation of the A^b-P/*a* heterozygote on the conservative side-chain hypothesis, with alpha and beta members occupying the same side chain. Alternative models shown here differ in regard to sequence of alpha and beta in the side branch, but in both cases the primary event leading to alpha isolation requires the participation of the homologue carrying recessive *a*. These diagrams show only one of the two duplicating strands, and coincidental backbone exchanges (secondary events), leading to various combinations of markers, are also omitted. For simplicity, the *sh* marker locus, which on this hypothesis would also occupy a side chain, is placed in the backbone.

beta segments in separate but adjacent side branches. On this model, spatial separation of these elements permits the isolation of alpha by a primary event (crossover) in the backbone (Figure 4A) and, since alpha can not be isolated by the reciprocal backbone event, this scheme accommodates the observation, to be noted in the next section, that one of the two types of alpha recombinants is far more frequent than the other. Assuming that the side branch carrying the recessive *a* allele may pair with either the beta or alpha segment, this model also permits isolation of alpha by two additional types of primary event, each involving the side chains of both homologues (Figures 4B and 4C). However, since these three possibilities for alpha isolation involve participation of both homologues in the *A* region, it is obvious that they can not explain the occurrence of

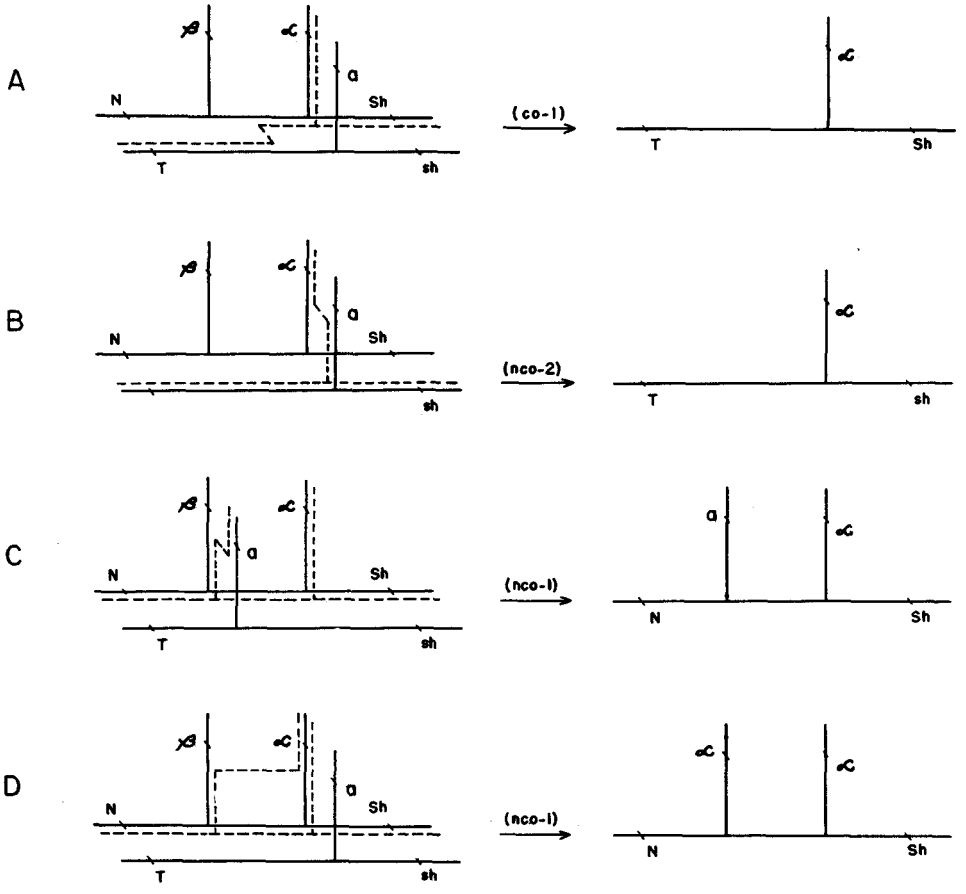


FIGURE 4.—Schematic representation of the A^b-P/*a* heterozygote on the modified side-chain hypothesis, with beta and alpha in adjacent side branches. Alpha isolation may occur by primary event in the backbone (A), or at the side-chain level (B, C, and D). Secondary events are not shown here, and only one of the duplicating strands is illustrated. As a matter of convenience, the *sh* marker is placed in the backbone rather than in a branch.

nonrecombinant alpha derivatives from the deficiency heterozygotes discussed above.

In fact, among the various mechanisms based on models presented here, only one (Figure 4D) will account for the occurrence of alpha derivatives without involving the homologue. It requires the assumption that the beta and alpha segments are homologous, and that copy-choice events (or exchanges) may take place between the adjacent beta and alpha side chains. Though this supposition may at first appear to be unwarranted, there is some justification for considering it a legitimate interpretation since there is independent evidence (LAUGHNAN 1955a,b) indicating that the beta and alpha members of both A^b-Ec and A^b-P are tandem, serial duplications. The event illustrated in Figure 4D would satisfy the evidence so far presented, since it is expected to yield nonrecombinant alpha isolations (α:α) by a mechanism not requiring participation of the homologue. However, this hypothesis may be considered valid only if the other types of alpha isolations illustrated in Figures 4A, 4B and 4C are realized among progeny of A^b-P/a heterozygotes.

To summarize then, since the multiple-exchange hypothesis and the conservative side-chain hypothesis require the involvement of the homologue in the primary event they are not adequate to explain the nonrecombinant alpha cases among the progeny of A^b-P/a-X and T-B3a hypoploid individuals, but this does not necessarily eliminate them as explanations for alpha cases from nondeficient A^b-P heterozygotes or homozygotes. The modified side-chain hypothesis, which allocates alpha and beta to separate side chains, will account for nonrecombinant alpha occurrences from the deficiency heterozygotes but is acceptable only if certain other types of alpha isolations, predicted on this model, are obtained from nondeficient heterozygotes. In the analysis which follows, data on alpha isolates from various A^b-P heterozygotes are considered for their bearing on the validity of these several hypotheses.

Evidence from A^b-*P heterozygotes*

The data reported in this section are from balanced heterozygotes in which the homologue of the A^b-P chromosome carries various other alleles. Unlike the hemizygotes dealt with in the foregoing section, these heterozygotes offer no impediment to occurrence of the primary event responsible for alpha isolation, and our concern here is with the distributions of marker combinations among alpha isolates and particularly with the fit of these distributions to predictions based on the proposed models.

Table 3 summarizes the data on alpha isolations from A^b-P/a heterozygotes marked in various combinations with *T* and *sh* and with *T* and *et*. The 358 alpha isolates from *T* and *sh* marked sources (data from *T* and *et* marked parents will be discussed at a later point) fall into only two of the four possible marker classes. The most frequent type (nco-1) carries the nonrecombinant markers of the parental A^b-P complex. The other type, designated co-1, carries the shrunken marker of the parental A^b-P chromosome and the *T* marking of its homologue; this recombinant accounts for over 20 percent of the alpha strands from A^b-Lima

TABLE 3

Constitutions of alpha-bearing strands from A^b-*P*/a *individuals marked with* T *and* sh *or with* T *and* et

T 7.1 β:α 0.25 *Sh* 12.8 *Et*

Source	A^b-P gametes tested	Number and distribution of alpha strands among offspring nco-1*	nco-2*	co-1*	co-2*
N A^b-Lima *Sh/T a sh*	30,390	20 *N* α *Sh*	0 *T* α *sh*	4 *T* α *Sh*	0 *N* α *sh*
N A^b-Lima *sh/T a Sh*	53,190	24	0	12	0
T A^b-Lima *Sh/N a sh*	462,810	185	0	46	0
Totals	546,390	229	0	62	0
N A^b-Cusco *Sh/T a sh*	38,410	14	0	3	0
N A^b-Cusco *sh/T a Sh*	56,950	13	0	22	0
T A^b-Cusco *Sh/N a sh*	1,750	1	0	1	0
T A^b-Cusco *sh/N a Sh*	18,760	6	0	7	0
Totals	115,870	34	0	33	0
T A^b-Lima *Et/N a et*	296,130	109	1	43	9

* As illustrated by the strand constitutions provided in the first row of this table, here and in similar tables to follow, nco-1 refers to a strand carrying the nonrecombinant markers of the parental A^b-P chromosome; nco-2 refers to a strand carrying the parental markers of its homologue; co-1 refers to a recombinant strand that carries the distal marker of the parental A^b-P chromosome and the proximal marker of its homologue; and co-2 refers to a recombinant strand that carries the proximal marker of the parental A^b-P chromosome and the distal marker of its homologue.

and for almost half of those from A^b-Cusco heterozygotes. Since the marked segment is less than eight units it is apparent that the recombinant event is not independent of the event that isolates alpha. Thus the alpha isolates of co-1 type are consistent with the argument that beta and alpha are left and right members respectively of the parental complex and that alpha may be isolated by a crossover (primary event), but this of course begs an explanation of the far more frequent nonrecombinant nco-1 type.

According to the multiple-exchange hypothesis which assumes that the primary event is a crossover between beta and alpha members (Figure 1A) the nco-1 type strand is the result of a second crossover to the left of beta (Figure 1B), thus reconstituting the parental marker combination. We note, however, that this model also calls for the occurrence of nco-2 type alpha isolations (Figure 1C) as a result of the alternative type of double exchange having the second crossover between α and *sh;* and co-2 alpha strands are expected to result from triple exchanges (Figure 1D). Yet neither of these types is represented in the data. It is interesting to note that in the investigations on the *ad8* cistron in Aspergillus (PRITCHARD 1960a,b), where the marked segments *y*–0.2–*ad*–6.0–*bi* are almost identical with the *T*–7.1–A^b–0.25–*sh* segments in maize, all four types of marker combinations were found among the adenine prototrophs and were attributed to single and multiple exchanges within a localized pairing region whose mean length is estimated to be 0.4 of a conventional map unit. It is conceivable that in maize there is a lower probability of exchange within the hypothetical localized pairing segment (PRITCHARD calculates 0.6 for the *ad8* region in Aspergillus). On this argument the absence of nco-2 and co-2 alpha strands is attributable

to the rarity of secondary crossover events in the relatively short *α-sh* segment. The data in the last row of Table 3 concerning alpha strands from *T* and *et* marked A^b-Lima parents are pertinent to this question since the near equality of the marked segments in this heterozygote (this time the distal segment is longer than the proximal) should, on the multiple-exchange hypothesis, lead to the isolation of equivalent numbers of double crossover nco-1 and nco-2 alpha strands. However, this expectation is not realized; the nco-1 type strands far exceed the nco-2 type, of which there is only one, and moreover, even the co-2 class, which on this hypothesis is attributable to a triple crossover, is more frequent than the nco-2 type.

Data from other types of A^b-P heterozygotes indicate that the distribution of recombinants among alpha progeny noted in Table 3 is not specifically identified with the A^b-P/*a* heterozygote. Table 4 summarizes the relevant data on strand constitutions of 27 alpha isolates from A^b-Lima and A^b-Cusco heterozygotes in which the homologue carries the standard *A* allele; and similar analyses of 78 alpha strands from heterozygotes in which isolated beta members (LAUGHNAN 1961) are carried in the homologue, are given in Table 5. Among the total of 105 alpha strands obtained in these two experiments, 81 are of the nco-1 type but there was not a single case of the nco-2 type nonrecombinant which, according to the multiple-exchange hypothesis, is expected to be equally frequent, or nearly so.

TABLE 4

Constitutions of alpha-bearing strands from A^b-*P*/A *individuals marked with* T *and* sh *or with* T *and* et

Source	A^b-P gametes tested	Number and distribution of alpha strands among offspring			
		nco-1	nco-2	co-1	co-2
N A^b-Lima *sh/T A Sh*	5,460	2	0	0	0
T A^b-Lima *Sh/N A sh*	19,225	4	0	1	0
N A^b-Cusco *sh/T A Sh*	22,600	9	0	2	0
N A^b-Lima *Et/T A et*	4,000	3	0	0	0
N A^b-Lima *et/T A Et*	550	2	0	0	0
T A^b-Lima *Et/N A et*	585	1	0	1	0
N A^b-Cusco *Et/T A et*	2,075	2	0	0	0
Totals	54,495	23	0	4	0

TABLE 5

Constitutions of alpha-bearing strands from T *and* sh *marked heterozygotes involving the* A^b-*Lima complex and isolated beta elements*

Source	A^b-P gametes tested	Number and distribution of alpha strands among offspring			
		nco-1	nco-2	co-1	co-2
T A^b-Lima *Sh/N* β-Lima *sh*	98,520	38	0	11	0
T A^b-*Lima Sh/N* β-Cusco *sh*	49,135	20	0	9	0
Totals	147,655	58	0	20	0

It is concluded that multiple exchanges within localized regions of pairing which we have seen cannot account for the nonrecombinant alpha isolations from hemizygotes, are also an unsatisfactory explanation for the occurrence of nonrecombinant alpha derivatives from balanced heterozygotes. It is obvious that recombination in the *A* region of chromosome 3 in maize does not follow the pattern of negative intereference which PRITCHARD (1960a) proposed for Aspergillus and has generalized to other organisms (1960b).

The hypothesis of localized pairing segments was suggested to account for the occurrence of apparent multiple exchanges (negative interference) in short segments. Since the data presented here indicate either an absence or extreme rarity of such multiple exchanges, there is reason to doubt that crossing over in maize takes place in localized regions of intimate pairing; the hypothesis will accommodate the data only if it is assumed that withing such a pairing segment there is sufficient positive interference to prevent the occurrence of more than a single exchange, and under these circumstances there is no compelling reason to favor the hypothesis as an alternative to the conventional view.

The conservative side-chain hypothesis, which we have seen can not account for alpha isolations from deficiency heterozygotes, predicts (Figure 3) that among the alpha progeny of A^b-P heterozygotes one of the two possible nonrecombinant types for markers should predominate. The data of Tables 3, 4, and 5 show an overwhelming majority of the nco-1 class and would seem to indicate that on this hypothesis the alpha locus is proximal to beta in the side chain (Figure 3A). Since on this model the primary event that isolates alpha is exclusively a side-chain phenomenon it is expected that the marker constitutions of alpha isolates will be determined solely by the pattern of coincidental backbone crossovers within the paired segment straddling the *A* locus. If this pairing segment is short relative to the marked segments, as assumed by the hypothesis of localized pairing, the distribution of these coincidental exchanges on either side of the point of attachment of the alpha-beta side chain is expected to be random; if, alternatively, the length of pairing segment approaches or exceeds the length of the marked segments themselves, as on the conventional view, the distribution of coincidental crossovers to left and right of the alpha-beta side chain should be proportional to the lengths of the marked segments themselves.

Inspection of the data in Tables 3, 4, and 5 indicates that neither of these predictions is satisfied. Considering only the recombinant classes for marker loci we note that the ratios for co-1:co-2 recombinants among alpha strands from *T* and *sh* marked A^b-Lima and A^b-Cusco parents, respectively, are 83:0 and 35:0, and that the corresponding ratio for *T* and *et* marked A^b-Lima individuals is 44:9. These results are clearly in conflict with the expectation, based on a restricted pairing segment, of near equality of the two classes. The data from *T* and *sh* marked parents are not in disagreement with predictions based on the assumption that all of the marked region is available for exchange since in this instance only 3.4 percent of the recombinants are expected to fall in the co-2 class (the *a–sh* map value, 0.25, represents 3.4 percent of the *T–sh* segment, 7.35). However, a similar calculation for the *T–et* segment leads to the expectation that 65 percent

of the recombinant alpha strands should be of the co-2 type (the *a–et* map value, 13.1, is about 65 percent of the *T–et* segment, 20.2). But the data in the last row of Table 3 are in disagreement with this prediction since only nine (17 percent) of the 52 recombinant strands are of the co-2 type. Thus, regardless of the length we assume for the effective pairing segment, the actual data on frequencies of recombination among alpha isolates are contradictory and lead to the conclusion that the conservative side-chain hypothesis is unacceptable.

As noted in a previous section, the modified side-chain hypothesis, which assumes that alpha and beta are carried on separate side chains, is the only scheme among the ones considered here that will satisfactorily account for nonrecombinant alpha occurrences from deficiency heterozygotes. Moreover, if the sequence of alpha and beta side chains is $T\ \beta{:}\alpha\ sh$, as represented in Figure 4, the occurrence of nco-1 and co-1 alpha recombinants from nondeficient A^b-P heterozygotes (Tables 3, 4, and 5) is anticipated; the former (nco-1) are interpretable as primarily the result of an event involving the beta and alpha side chains of the A^b homologue (Figure 4D), whereas the latter (co-1) are expected to result primarily from backbone crossovers of one type (Figure 4A) between the beta and alpha side chains. However, according to this scheme, A^b-P heterozygotes are expected to yield alpha derivatives as a result of two additional primary events, both occurring at the side-chain level. One of these involves a switch in copy (or exchange) between homologous side chains carrying the beta and the recessive *a* elements (Figure 4C) and is expected to yield occasional nco-1 strands carrying the $a{:}\alpha$ type complex; the other involves the alpha side chain in a corresponding event and leads to isolations of alpha in nco-2 type strands (Figure 4B). The data are clearly not in agreement with the latter prediction since among the 625 alpha isolations reported in Tables 3, 4, and 5, only one nco-2 type strand was isolated and this occurred among the progeny of a *T* and *et* marked parent. The $a{:}\alpha$ type complex, whose isolation in nco-1 type strands is anticipated from the alternative event, should exhibit the dotted type of mutability since it carries the recessive *a* allele which mutates to *A* in the presence of *Dt* (RHOADES 1938). Mutable alpha derivatives have been identified but have occurred almost exclusively among the co-1 class, indicating that the occurrence of the $a{:}\alpha$ complex is attributable to conventional crossing over rather than to side-chain events.

Purely hypothetical considerations (WINGE 1955; SCHWARTZ 1955; TAYLOR 1957) suggest an endless array of possible variations on the branched chromosome model. If, however, the two alternative schemes treated here may be considered to incorporate the basic recombinational features of side-chain models in general, the data justify the conclusion that the anomalous, nonrecombinant alpha isolations are not attributable to primary events in side chains. Indeed, the evidence from these studies is opposed to the view that genetic material in maize is located in branches of the chromosome that offer the opportunity for recombination at that level.

Evidence from inversion-3a heterozygotes

These studies deal exclusively with alpha derivatives from inversion hetero-

zygotes in which one chromosome 3 member, either normal or interchanged, carries the beta:alpha complex and the other carries the recessive *a* allele in the differential segment of the paracentric inversion-3a. The breakpoints of this inversion have been placed on the cytological map of the long arm of chromosome 3 at 3L 0.4 and 3L 0.95 (RHOADES and DEMPSEY 1953; personal communication); the proximal point of exchange is to the left of lg_2 (liguleless-2) and the distal break is to the right of *et*, such that the *lg*, *T*, *a*, *sh* and *et* loci are all within the inverted segment which includes a minimum of 61 units of the genetic map. The distal breakpoint of this inversion is about 15 map units beyond *A*, which means that this segment represents about one fourth of the genetic length of the inversion. As expected, balanced products of single crossovers within the inverted segment are not found among the backcross progeny of plants heterozygous for this inversion, but double crossover strands are recovered in 1.1 percent of functional male gametophytes and in 1.8 percent of functional female gametophytes (RHOADES and DEMPSEY 1953).

Inversion-3a heterozygotes offer an ideal opportunity to test the hypothesis of multiple exchange since strands involved in a single exchange or in odd numbers of exchanges within the inverted segment of these heterozygotes will not be represented among the offspring. Accordingly, the products of single exchanges between the beta and alpha members, which ordinarily would be scored as recombinant alpha strands, should be eliminated from the progeny of these individuals. But according to the hypothesis of localized pairing, which would account for the alpha nonrecombinant class as the result of double exchanges in a short, effectively paired segment spanning the *A* locus, the A^b-P inversion heterozygotes should yield nonrecombinant alpha progeny. The expected distribution of these nonrecombinant alpha strands may be deduced by a consideration of the events illustrated in Figure 5 which gives a diagrammatic representation of the inversion heterozygote in which the beta:alpha complex is carried in a noninterchanged

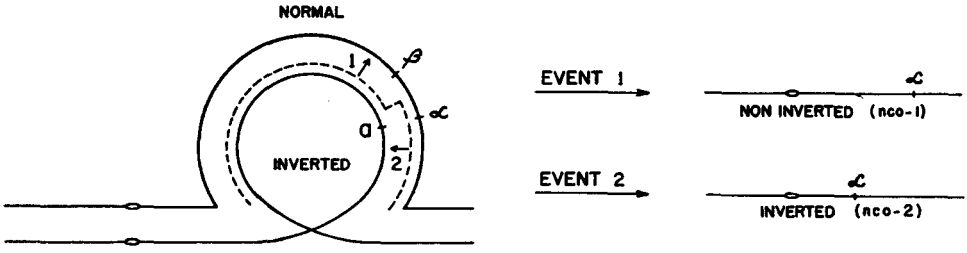


FIGURE 5.—Pairing configuration in the heterozygous, paracentric inversion-3a individual with the β:α complex in the normal chromosome and recessive *a* in the inverted homologue. A primary event which isolates alpha is illustrated by the dashed strand, and additional exchanges involving this strand and occurring either to the left (event 1) or to the right (event 2) are illustrated with arrows. If, as on the multiple-exchange hypothesis, the effective pairing region is short, events 1 and 2 are expected with equal frequencies and should lead to the isolation of equivalent numbers of nco-1 and nco-2 alpha isolations shown at the right. For the sake of simplicity the exchanges are diagrammed as copy choice events but the predictions are the same if exchanges between previously duplicated strands are assumed.

chromosome 3. Assuming the initial occurrence (primary event) of a switch in copy (or an exchange) between beta and alpha, a second such event within the differential segment may be either to the left (event 1) or right (event 2) of the A^b complex. As shown in Figure 5, the first of these is expected to isolate alpha in a noninverted strand (nco-1) whereas the second should lead to alpha isolations in the inverted homologue (nco-2). If, according to the multiple-exchange hypothesis, the effective pairing region is short relative to the segments located to the left and right of the complex and within the inversion, these two types of nonrecombinant alpha strands are expected to be equally frequent. However, if the frequencies of the two types of secondary crossovers are held to be proportional to the lengths of the marked segments within which they may occur, the nco-1 type alpha strand is expected to be three or four times more frequent than the nco-2 type; and if account is taken of the likely effect of chromosomal interference the discrepancy between the frequencies of these two types should be even greater.

The distributions of alpha derivatives among backcross progenies of A^b-P inversion heterozygotes are given in Table 6. It should be noted that the allocation of an alpha strand to the nco-1 or nco-2 class in this instance does not require gene markers since there is no difficulty in making this determination on the basis of pollen abortion in the two types; thus, individuals which are heterozygous for an alpha-carrying strand of nco-2 type (inverted) and a normal *a* strand contributed by the tester parent, should regularly exhibit from 15 to 20 percent pollen abortion, while the nco-1 type plant, which carries two normal chromosomes 3, is expected to have normal pollen. The data in the first two rows of Table 6, which deal with alpha strands derived from inversion heterozygotes in which the A^b-Lima complex is carried in a noninterchanged chromosome, are not in agreement with the multiple exchange hypothesis since there were 67 isolations of the nco-1 type and not a single case of the nco-2 type alpha strand predicted on this model. However, even though there were no nco-2 type alpha derivatives among the progeny of these inversion heterozygotes, it is not unlikely that some of the nco-1 alpha isolates represent double crossovers of the conventional type

TABLE 6

Alpha derivatives from heterozygotes carrying A^b-*P in a normal or interchanged chromosome and recessive* a *in the inverted segment of Inversion-3a*

Source	A^b-P gametes tested	Distribution of alpha strands among offspring: Noninverted (nco-1)	Inverted (nco-2)
N A^b-Lima/In 3a: *a*	51,930	56 *N* α	0
N A^b-Lima *et*/In 3a: *a Et*	8,400	11 *N* α *et*	0
T A^b-Lima/In 3a: *a**	26,880	12 *T* α	0
T A^b-Lima *et*/In 3a: *a Et*	17,060	7 *T* α *et*	0
T A^b-Lima *sh*/In 3a: *a Sh*	4,220	2 *T* α *sh*	0
T A^b-Cusco *sh*/In 3a: *a Sh*	37,870	23 *T* α *sh*	0

* In this and the three following heterozygotes, the A^b-P complex is carried in a 2^3 interchanged, noninverted chromosome, and recessive *a* is carried in the inverted, noninterchanged chromosome 3.

since there is a relatively long region to the left of the primary event, but still within the differential segment of the inversion, available for the secondary event.

The frequency of nonrecombinant alpha occurrences (*ca.* one per 900 tested gametes) from these inversion heterozygotes is higher than that for any genotype we have tested so far. Since it may be calculated from data in the original report (RHOADES and DEMPSEY 1953) that double crossovers within the differential segment of inversion-3a heterozygotes are realized with a frequency only about one fifth of that expected for corresponding events in noninversion heterozygotes, the observed high frequency of alpha occurrences in these studies is itself at odds with the argument that the nonrecombinant alpha occurrences are primarily attributable to double crossovers of the conventional type.

The data in the last four rows of Table 6 may be taken as further evidence against the multiple-exchange hypothesis. The alpha strands recovered here derive from inversion heterozygotes in which the A^b complex is carried in an interchanged 2^3, noninverted chromosome. If it is assumed that effective pairing segments are highly localized, consideration of the rather complicated configuration expected in these heterozygotes leads to the expectation of equal numbers of nco-1 (50 percent aborted pollen in this instance) and nco-2 (15 to 20 percent aborted pollen) alpha derivatives. And if the effective pairing segment is relatively extended, as on the conventional view, it can be shown that these heterozygotes should still yield nearly equivalent numbers of alpha strands of these two types, in addition to a third class carrying alpha on a reconstituted normal chromosome 3. However, the pertinent data in Table 6 indicate that alpha isolations from these heterozygotes occur exclusively in association with nco-1 type strands.

These observations support the conclusion, reached on the basis of evidence from hemizygotes and balanced heterozygotes, that the hypothesis of multiple exchange in localized regions of pairing is not a satisfactory explanation for the occurrence of the nonrecombinant alpha derivatives. Beyond this, the evidence from inversion heterozygotes suggests that the mechanism involved in the isolation of nonrecombinant alpha derivatives does not require exchange between homologues, a conclusion which was first suggested by the evidence from deficiency heterozygotes.

The data on alpha isolations from inversion heterozygotes should be useful in testing predictions based on the side-chain hypotheses. We presume that the backbone of the chromosome, but not the side chain, is involved in the breaks and rejoins required to produce an inversion and that the complications which arise following certain backbone events in individuals heterozygous for the inversion should not attend the hypothetical occurrences that we pose at the level of the side branches. Thus, while the vast majority of backbone crossovers occurring in the differential segment of inversion heterozygotes should not be realized among their progeny, the products of hypothetical side-chain events from these same individuals should not be reduced in frequency among the offspring, except to the extent that (a) mechanical hindrance to pairing in the inversion

heterozygote may reduce the initial frequency of side-chain events between homologues, and (b) the side-chain event is correlated positively with backbone events which would lead to disproportionate elimination of the former from the progeny.

Referring once more to Figure 3A we note that on the conservative side-chain hypothesis, the primary event isolates alpha on the side chain of a strand carrying the parental markers of the A^b-P chromosome (nco-1). Since, on this model, alpha and beta reside in the same branch only one type of primary event is possible. In inversion heterozygotes of the type illustrated in Figure 5 this event should lead to isolations predominantly of the nco-1 type in which the alpha element is carried in a normal chromosome. Alpha strands with single coincidental crossovers in the inversion would be eliminated and the only basis for the isolation of alpha in the inverted homologue (nco-2) would be a coincidental double crossover in adjacent regions of the backbone. While, except for the absence of the nco-2 class, the data given in Table 6 are not seriously in conflict with this scheme, it should be recognized that the test involved here is not an exacting one and that the more critical evidence from hemizygotes and balanced heterozygotes could not be accommodated on this hypothesis. Moreover, it is significant to note (see Figure 3A) that if the a member is assumed to pair indiscriminately with alpha or beta in the homologous side branch, the side-chain event in the latter instance should isolate an $\alpha{:}a$ complex which, as we have already seen, is expected to mutate in response to the *Dt* gene. Forty-seven of the 111 alpha isolations from inversion heterozygotes listed in Table 6 have been tested for *Dt*-induced mutability and all but one of these proved to be stable. The single mutable case may be considered the result of a primary crossover event isolating the $a{:}\alpha$ complex, accompanied by a coincidental exchange to reconstitute the nco-1 strand type.

The data from inversion heterozygotes afford a critical test of the modified side-chain hypothesis since according to this scheme side-chain events (Figures 4B, 4C, and 4D) in such heterozygotes (see Figure 5) should lead not only to $\alpha{:}\alpha$ and $a{:}\alpha$ isolates in the noninverted (nco-1) chromosome but also to nco-2 type derivatives in which alpha is carried in the inverted homologue. However, these predictions are not realized since, as already noted, nco-2 type alpha isolates are not represented among the progeny of inversion heterozygotes (Table 6) that produced 111 nco-1 type alpha derivatives. Moreover, as noted above, mutability tests of 47 of these derivatives indicate that only one is likely to have the constitution $a{:}\alpha$; as pointed out in the preceding paragraph, this case may be explained on conventional grounds as the result of a double crossover.

To summarize, the evidence from inversion heterozygotes, like that from hemizygotes and balanced heterozygotes, indicates that neither the multiple-exchange hypothesis nor the side-chain hypothesis is adequate to account for the origin of nonrecombinant alpha derivatives from the A^b-P complexes.

DISCUSSION

The recent impetus lent to both the multiple-exchange and side-chain hypotheses clearly derives from the need to explain the seemingly contradictory, but

not infrequently encountered, evidence from several microorganisms that prototrophs, expected to arise as a result of crossover events, are too frequently isolated as nonrecombinants for marker loci. The experiments described here were undertaken to determine whether the nonrecombinant alpha derivatives from A^b-P complexes, which show similar deviations from the expected pattern, might reasonably be attributed to either of these mechanisms. The evidence leads to the conclusion that neither of these mechanisms will satisfactorily account for the exceptional alpha isolations. The writer is strongly inclined toward the further conclusion, which is not identical with the first, that the phenomena in question do not occur in maize, but it is apparent that certain considerations, aside from the one that these studies involve only one segment of the maize genome, argue in favor of a less sweeping statement.

The data from A^b-P/a-X hemizygotes and from the T-B hypoploid indicate that isolation of the alpha component is possible without participation of the homologue. The only reasonable conclusion to be reached from this finding is that some kind of intrachromosomal mechanism is involved in these alpha occurrences. However, unless the assumption is made that nonrecombinant alpha strands arise by only one mechanism, the evidence from the deficiency heterozygotes has no critical bearing on the validity of the multiple-exchange and side-chain hypotheses, since, assuming a multiple origin, this background would screen effectively against cases originating by either of these latter mechanisms, but would not be expected to eliminate, or even diminish, those cases arising by the intrachromosomal event. In this connection it should be noted that the frequency of nonrecombinant alpha occurrences is at least as high among the progeny of hemizygotes as among the offspring of sib, balanced heterozygotes (LAUGHNAN 1961). This observation favors a single origin (intrachromosomal) for these derivatives but, so far as the deficiency experiments are concerned, may be regarded as circumstantial evidence only since it could be argued that some kind of compensating influence is involved in the multiple origin of alpha exceptions.

Unlike the deficiency heterozygotes, the balanced A^b-P heterozygotes and the A^b-P inversion heterozygotes should pose little or no hindrance to the primary alpha-isolating events assumed for either the multiple-exchange hypothesis or the side-chain models. Nevertheless, the distribution of recombinant and nonrecombinant alpha strands from these backgrounds is inconsistent with the patterns predicted by these schemes, and the conclusion is warranted that they are not involved in the origin of the anomalous alpha derivatives.

It is perhaps more significant to note that if the phenomena of multiple exchange (high negative interference) and/or of recombination between hypothetical side branches of the chromosome occur in maize, certain critical types of alpha strands should be obtained among the progeny of the balanced and inversion heterozygotes. Since these were not realized we may conclude that (a) multiple exchanges within short chromosomal segments either do not occur in maize or occur so rarely that they were not apparent in the studies reported here, and (b) the assumption that the genetic material in maize, including the alpha

and beta elements, is oriented in side chains that represent lateral extensions of the chromosome backbone and are subject to recombinational events *inter se*, is untenable.

It is conceivable that the phenomenon of localized pairing may exist in maize even though there is no compelling reason, from the studies reported here, to assume it. PRITCHARD's analyses of recombination within the *ad8* cistron and nearby segments in Aspergillus, and his conclusions from these studies, may suggest that the hypothesis of localized pairing should not be abandoned at this time for maize even though it appears that in this material multiple exchanges are not realized within the hypothetical effectively paired region.

Occasional reference has been made in this presentation to an intrachromosomal mechanism involved in the origin of alpha nonrecombinants. The evidence on alpha derivatives from the deficiency heterozygotes clearly indicates that such a phenomenon is involved, and the results obtained from the balanced and inversion heterozygotes are consistent with this conclusion. Preliminary consideration has been given to such a mechanism in other publications (LAUGHNAN 1955c, 1957, 1961) and a detailed treatment is in preparation at this time.

SUMMARY

A detailed analysis of the distributions of alpha-carrying strands among the progeny of A^b-P parents carrying the beta:alpha complex was undertaken. In particular, the fit of these distributions to expectations on the hypothesis of multiple exchange in localized regions of pairing, and on two alternative side-chain models, was investigated.

The yield of alpha derivatives from A^b-P/*a*-X deficiency heterozygotes and from T-B hypoploid individuals indicates that isolation of alpha from the beta:alpha complex does not require participation of the homologue, and indicates that the origin of the vast majority of alpha nonrecombinants is attributable to an intrachromosomal mechanism.

The distributions of recombinant and nonrecombinant alpha strands from various balanced A^b-P heterozygotes, and from individuals carrying the A^b-P complex in a normal or interchanged chromosome and the recessive *a* allele in an inversion-3a homologue, are inconsistent with expectations on both the multiple-exchange hypothesis and the side-chain models.

It is concluded that (a) multiple exchanges within short chromosomal segments either do not occur in maize or occur so rarely that they were not apparent in the studies reported here, and (b) the assumption that alpha and beta, and presumably the genetic material in general in maize, are oriented in side chains that are subject to recombinational events is untenable.

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