BEHAVIOUR OF FLANKING MARKERS IN ALLELIC CROSSES

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Summary

The principles of analysis of the distributions of flanking markers amongst prototrophic recombinants arising from allelic auxotrophic differences are discussed. Considerable variations occur in the parameters which may be measured and also in the degree of agreement of criteria used to place allelic differences in order in fine structure maps. At least some of these variations appear likely to depend upon genetic differences affecting the mechanisms which result in genetic recombination and the mechanisms which control their activity. An approach to a quantitative theory of the distribution of flanking markers is suggested.

I. Introduction

The progenies of crosses between alleles provide data for the fine structure mapping of gene loci and for the study of the mechanism of recombination. At the present time the latter is an important unsolved problem. It is a well-tried principle that the analysis of genetic variation is a potent means of discovering the components of a physiological process.

Allelic crosses yield data on the frequency of prototrophs. It is known already that this parameter is subject to wide variation due to genetic causes. A recessive allele (*rec–1*) of one of these genetic factors results in a great increase in frequency of prototrophs if it is contributed to the cross by both parents (Jessop and Catcheside 1965). The factor of increase is commonly about 15–20 or more. However, it now seems that there are several other genetic factors which also affect the frequency of prototrophs, but their individual effects and modes of interaction are not yet understood.

Allelic crosses also yield data upon how the parts of the parental chromosomes on the flanks of the alleles are represented amongst the prototrophs. These parts of the parental chromosomes are marked, for experimental purposes, by suitable genes, normally at one locus on each flank of the locus whose allelic recombination is being studied. Hence, amongst the resulting prototrophs, four combinations of flanking markers may occur. This discussion will be concerned with the variation of the frequencies with which these four combinations occur and how study of the variation may contribute to an understanding of the mechanism of genetic recombination.

The closeness of linkage of the flanking markers used to study any particular locus depends upon what markers are available. They must not interfere with the scoring of one another, nor with genes at the locus they flank. In most cases, convenient markers occur between 1 and 10 cross over units from the locus at which

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allelic recombination is studied. However, it must be noted that the cross over value between any two loci is not an absolute quantity, but may be affected by alterations in the genetic background. The value can be changed in this way by more than one order of magnitude.

II. Analytical Procedures

In a cross of the general type $K\ m^1\ L \times k\ m^2\ l$, where m^1 and m^2 are alleles whose recombination is being studied, while K and k and L and l are, respectively, the proximal and distal flanking markers, selection is made for m^+ prototrophs and these are classified into the four groups $K\ L$, $k\ l$, $K\ l$, and $k\ L$. These data have often been used to deduce the order of the alleles m^1 and m^2 with respect to the K and L loci. Characteristically, at least in *Neurospora*, *Aspergillus*, and maize, all four classes occur with substantial frequencies and there is no overwhelming representation of one of the classes, as occurs when the two middle genetic differences of a four-point test are closely linked nonallelic genes. However, the data have been treated usually as though the event of recombination between m^1 and m^2 were totally equivalent to that between two nonallelic genes. For the purpose of deducing an order, it is not necessary to assume this. Mutually consistent rules may be drawn up and stated as follows. The rules are empirical and mutally consistent in that I is a compound of II and III; their equivalence to rules which would follow from orthodox crossing over does not imply acceptance of the view that the distribution of flanking markers amongst allelic recombinants is due to orthodox crossing over. The only other set of mutually consistent rules would be the converse of those stated.

I. If the recombinant classes are compared, the order will be $K\ m^1\ m^2\ L$ if $k\ L$ is in the majority and will be $K\ m^2\ m^1\ L$ if $K\ l$ is in the majority.

II. If the proximal flanking alleles only are considered, the order will be $K\ m^1\ m^2$ if k is in the majority and will be $K\ m^2\ m^1$ if K is in the majority. In other words, the more frequent proximal flanking allele identifies the more distal of the m allele differences, because they entered the cross together.

III. If the distal flanking alleles only are considered, the order will be $m^1\ m^2\ L$ if L is in the majority and will be $m^2\ m^1\ L$ if l is in the majority. That is, the more frequent distal flanking allele identifies the more proximal of the m allele differences, since they entered the cross together.

The first rule uses only the recombinant classes, whereas the second and third rules also use the parental classes, which together often amount to half of a progeny. Hence, although the second and third rules are not wholly independent of the first, they have the merit of using all of the progeny scored. Indeed, as will be seen, the frequencies of the proximal and distal markers are virtually uncorrelated amongst the four classes of the flanking markers, so that rule I provides almost no information not provided by the other two. Moreover, in some situations, it may be technically possible to provide only a proximal or a distal flanking marker.

Since rule I is a compound of rules II and III, it is convenient to adopt the convention of regarding rule I as the master one in any case where conflict occurs between the orders indicated by the three rules. The inequalities of the data used in

rules II and III are compounded in those used in rule I. However, rules II and III conflict in some cases; in these, that with the more unequal representation of flanking markers will dominate in the application of rule I. The situation is not entirely satisfactory, nor can any more rational approach be suggested until much more is known about the mechanism of allelic recombination and of the factors leading to the observed distributions of flanking markers.

One of the forms of variation encountered in the data is the agreement or disagreement of the orders of m^1 and m^2, with respect to K and L, deduced using each of the rules.

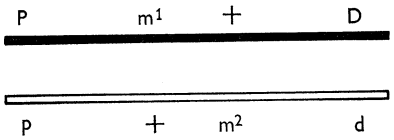

Fig. 1.—Diagram of the conventions adopted for labelling flanking markers with respect to a pair of allelic differences between them.

For consideration of the variation of the frequencies of the respective flanking markers it is convenient to express the data in common terms, without regard to the particular flanking allele which is predominant. By such means reciprocal crosses and different sets of data may be compared directly. It is proposed to express all data in terms of the formulation of Figure 1. In this formulation, the proximal markers are labelled P and p, while the distal markers are labelled D and d. The proximal and distal markers on the parental chromosome which carries the proximal allele difference, m^1, are given capital letters, P and D. The flanking markers on the parental chromosome which carries the distal allele difference, m^2, are given lower case letters, p and d.

Of course, some difficulties arise in extensive data. The most important are direct contradications between different sets of data involving the same genetic markers in the same arrangement. An example is to be seen in Table 1 in the fifth and sixth sets of data for the *his–1* locus. The entries in Table 1 for all six *his–1* crosses are set out as though the order *am* K83 K651 *inos* were the same for all; the sixth entry, however, points to the order *am* K651 K83 *inos*.

Admitting the difficulties, data can be expressed algebraically in consistent terms. Let x be the chance that a given prototroph is P and $(1-x)$ that it is p. Let y be the chance that a given prototroph is D and $(1-y)$ that it is d. The algebraic frequencies of the four classes of prototrophs would then be:

Class	PD	pd	pD	Pd
Frequency	xy	$(1-x)(1-y)$	$(1-x)y$	$x(1-y)$

Comparisons of the expected numbers in the four classes, calculated using the estimates of x and y obtained from the data, show very good agreement with observation. No other parameter appears necessary to allow for possible correlation in the combination of the probabilities of occurrence of the flanking markers.

TABLE 1

DISTRIBUTION OF FLANKING MARKERS AMONGST PROTOTROPHS FORMED BY ALLELIC AND NONALLELIC RECOMBINATION IN *NEUROSPORA*, *ASPERGILLUS*, AND MAIZE

In the first column, the alleles are given in the order (distal on the left) deduced by application of criterion I (see text). Asterisks mark differences significant at least at the 5% level

Locus	Flankers		Distribution of Flanking Marker Combinations amongst Prototrophs				Flanking Markers Taken Individually				x	y	Source of Data
	P/p	*D/d*	*PD*	*pd*	*Pd*	*pD*	*P*	*p*	*D*	*d*			
					Allelic recombination								
Neurospora													
his-1 (K83, K651)	*am*^{6}/+	+/*inos*	112	134	86	93	198	227	205	220	0·47	0·48	Catcheside (unpublished
			64	57	51	67	115	124	131	108	0·48	0·55	data)
			75	78	55	79	130	157	154	133	0·45	0·54	
			65	66	42 *	92	107 *	158	157 *	108	0·40	0·59	
			6	17	7	18	13 *	35	24	24	0·27	0·50	
	am^1/+	+/*inos*	19	8	15	6	34 *	14	25	23	0·71	0·52	
me-2 (K23, P2)	+/*try-4*	*pan-1*/+	12	54	15 *	32	27 *	86	44 *	69	0·24	0·39	Murray (1963)
his-5 (K516, K548)	+/*pyr-3*	*leu-2*/+	24	145	19 *	219	43 *	364	243 *	164	0·11	0·60	Smith (1965)
Aspergillus													
pab-1 (5, 6)	+/*ad-9*	+/*y*	18	53	9 *	107	27 *	160	125 *	62	0·14	0·67	Siddiqui (1962)
Maize													
wx (90, Coe)	+/*v*	+/*bz*	15	27	3 *	63	18 *	90	78 *	30	0·17	0·72	Nelson (1962)
					Nonallelic recombination								
Neurospora													
ad-3A/ad-3B	*his-2*/+	*nic*/+	6	5	0 *	526	6 *	531	532 *	5	0·01	0·99	Giles, de Serres, and Barbour (1957)

The formulation is equivalent to assuming that x is the frequency of crossing over between P and m^1 and that $(1-y)$ is the frequency of crossing over between m^2 and D. The advantage of stating the matter in the terms suggested is that it avoids direct ascription to conventional crossing over and also the overtones of "negative interference" which merely describes the situation in terms of an unsuitable theory.

If the formulation were completely consistent with the three rules stated above, the following directional inequalities would be expected, corresponding to the three rules:

Rule I	$(1-x)y > x(1-y)$
Rule II	$(1-x) > x$
Rule III	$y > (1-y)$

Departures from these relations of inequality would result from disagreement between the orders of the allelic differences deduced from the rules. Consistency requires x to be less than a half, while y is greater than a half. Values of x and y are entered, in Table 1, in two columns towards the right-hand side.

The distributions of the proximal and distal flanking markers may be represented diagrammatically by plotting the square roots of the numbers of P against those of p and, likewise, those of D and d against one another. This has the advantage of displaying the data so that their statistical consistency, or lack of it, may be seen at a glance. For consistency, about 95% of all points should lie within a band 2 units wide, the unit being the distance between two successive whole number roots on the ordinate or abscissa, i.e. the difference between $\sqrt{n^2}$ and $\sqrt{(n+1)^2}$, where n^2 and $(n+1)^2$ are the numbers of individuals in a class. Plots of data (Fig. 2) for the *Neurospora* genes *his–5* (Smith 1965) and *me–2* (Murray 1963) and for the *Aspergillus* gene *pab* (Siddiqui 1962) show degrees of scatter which imply considerable heterogeneity within the data. Comparison of values of x and y with the genetic maps of these genes has shown no evidence that their variation is related in any way to the positions of the alleles.

III. Flanking Marker Distributions

Samples of data, drawn from *Neurospora*, *Aspergillus*, and maize, are given in Table 1.

It is characteristic for each of the four classes to be fairly well represented. The *Pd* class, in particular, which would correspond to a triple cross over if allelic recombination really occurred by a classical cross over, is quite frequent, sometimes amounting to a fifth of all prototrophs (*his–1*, Table 1). This is in marked contrast to what is found if the central genetic differences involve nonallelic genes, as in the data of Giles, de Serres, and Barbour (1957) on *ad–3* (Table 1). These *ad–3* data show the relations expected on the classical principles of crossing over.

The complex of events which results in allelic recombination is presumed to be identical to the complex which, conditionally, results in nonallelic recombination. If so, the way the flanking markers are distributed should throw light on the mechanism. Observations show that the distributions show considerable diversity both within and between species. How the variations may be explained is considered in the discussion.

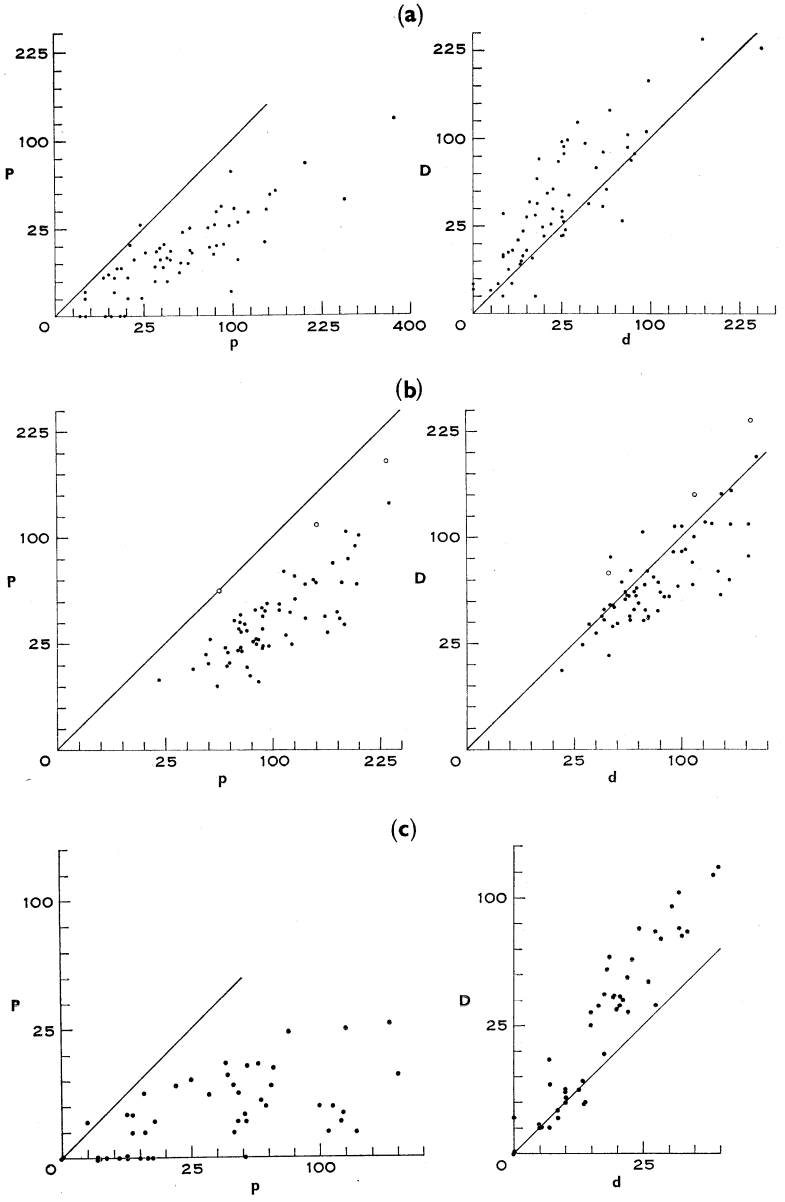

Fig. 2.—Square root charts demonstrating the relative frequencies of proximal (P, p) and distal (D, d) flanking markers. The sloping line is at an angle of 45°, representing equality of the alleles in any sample. (*a*) *Neurospora crassa his–5* data of Smith (1965). (*b*) *Neurospora crassa me–2* data of Murray (1963). (*c*) *Aspergillus nidulans pab–1* data of Siddiqui (1962). The wide scatter shows that there is considerable heterogeneity.

The order m^1m^2, of the alleles, is usually based on the *pD* class of recombinants being more numerous than the *Pd* class. The data in Table 1 exhibit a considerable range in the degree of difference between *Pd* and *pD* and it would be possible to cite data in which these classes were even nearer to equality. The variation shown is not due, at least in *Neurospora*, to the particular genetic regions studied, but rather to the fact that the stocks used differ in genes concerned with recombination. This is made clear by the differences shown by crosses of different stocks of the same genes, especially the data on the *his–1* alleles K83 and K651.

The data also exhibit a wide variation in the proportion of the prototrophs which belong to the recombinant classes (*Pd*+*pD*). The proportion ranges from less than a half to about three-fifths. Table 1 also shows variation in respect of the proportions of *PD* and *pd*. Likewise there is marked variation in the values of x and y, especially the former. No information is yet available about the genetic determination of the differences observed.

IV. Order of Alleles and Agreement between Criteria

Data of Case and Giles (1958) relating to *pan–2* alleles, flanked proximally by *ylo* (or *ad*) and distally by *try–2*, include 27 crosses each of which gave a substantial number of prototrophs. Seven of these were crosses involving the same pair of alleles, *B3* and *B5*, but no two were exact replicates. Five stocks of *B3* and three of *B5* were involved in the crosses. In 26 crosses, the order of the *pan–2* alleles deduced from rules I and II agrees; in 18 cases the order is based on numerical differences which are significant at least at the 5% level. In the one case of disagreement (*ylo B25*$\times$ *B10 try–2* in Table 12 of Case and Giles 1958), the orders are based on differences which are insignificant; in this case the order based on rule III agrees with that from rule I, itself consistent with the reciprocal cross. Indeed, the application of rule III yields only one disagreement with the order from rule I (*ylo B9*$\times$*B10 try–2* in Table 10 of Case and Giles 1958), but in this case also the differences are insignificant. Altogether, 19 of the orders based on rule III are derived from data in which the differences are significant at least at the 5% level.

The values of the parameters x and y vary quite widely, but in the case of the two disagreements noted above one or other parameter was very close to 0·5. In each case, this occurred in the one which disagreed with the other two. For these *pan–2* data, as a whole, it can be said that the various criteria used to order the alleles agree completely.

The data on *his–5* (Smith 1965) also show concordance of orders deduced by application of each of the three criteria. The few exceptions are based either on small numbers of prototrophs or on insignificant differences.

The data of Murray (1963) present a complete contrast. The major part of her excellent data, relating to *me–2* alleles, flanked proximally by *try–4* and distally by *pan–1*, is in her Table 4. This contains the results of 53 crosses, many of them representing reciprocals. The orders based on rules I and II agree for every one of the 53 crosses; in 36 of the crosses the differences upon which the orders are based are significant at least at the 5% level. In contrast, only 10 of the orders based on rule III

agree with the order based on rules I and II. Altogether, 21 of the orders based on rule III are founded on differences which are significant at the 5% level. Only one of these (*try–4* P81 × K98 *pan–1*) agrees with the order based on rules I and II; the others all disagree. A similar situation holds for the eight crosses given in Murray's Table 6, although none of the orders based on rule III are founded on significant differences.

The three crosses in Murray's Table 7 provide a contrast with all of her other crosses, except for the instance previously noted. The anomaly of these crosses is that they agree with the consistent situation represented in the *pan–2* data of Case and Giles (1958). In this case the values of x and $(1-y)$ are generally less than $0{\cdot}5$, though for several the values are not significantly different from $0{\cdot}5$. Most of Murray's *me–2* data have x less than $0{\cdot}5$ and $(1-y)$ greater than, or approximately equal to, $0{\cdot}5$. However, the three "anomalous" crosses in her Table 7, as well as the *try–1* P81 × K98 *pan–1* cross in her Table 4, have $(1-y)$ less than $0{\cdot}5$ with x less than or, in one case, equal to $0{\cdot}5$. These statements about the values of x and $(1-y)$ depend upon the convention of regarding criterion I as the master one.

The fact that the two situations, of agreement and disagreement of order deduced from rules II and III, can occur in the same set of data, suggests a genetic difference in respect of one of the functions making up the process of allelic recombination. The *me–2* alleles themselves do not seem separable into two special categories, even though P81 happens to figure in each of the four *me–2* crosses which are unlike all the others. The published data do not allow the relationships of the various stocks used to be traced.

Stadler and Towe (1963) give data in their Table 1 for allelic recombination between *cys* mutants using *ylo* as proximal and *lys* as distal flanking marker. There are 26 crosses; the orders of the *cys* alleles, deduced from rules I and II, agree in 22 cases. The orders deduced from rules II and III agree in only 10 cases. In three cases, out of four in which the distal flanking marker data show a highly significant inequality, the rule II and rule III orders disagree.

A similar situation is found in the data of Jessop and Catcheside (1965) for allelic recombination between *his–1* mutants using *am* as proximal and *inos* as distal flanking markers. Generally the orders deduced from rules I and III agree, but the order from rule II rather often disagrees with the other two. In 35 crosses having sufficiently abundant data, such disagreement is shown in 12 cases. In the eight cases where the rule I and III orders disagree, the rule II and III orders also disagree. Altogether, the orders based on rules II and III disagree in 18 cases, four of these having highly significant differences in representation of the respective flanking markers.

Further extensive analysis of crosses between numerous different stocks of the *his–1* alleles K83 and K651 has disclosed substantial variation in the frequencies of representation of the flanking markers *am* and *inos*, especially the former, amongst *his–1*$^+$ prototrophs. Likewise similar variation in the distribution of *his–1* has been found amongst *am*$^+$ prototrophs in crosses involving *am* alleles. Nothing is yet known about the genetics of such variation.

V. Discussion

Proof that the genetic material of the chromosome is DNA raised new problems in the comprehension of genetic crossing over. Instead of having single threads to cut and rejoin, as in the diagrams of classical formal genetics, each chromatid at meiosis consists of a DNA molecule, which is made of two strands bonded together. If it were true, as some think, that each chromatid consists of several DNA molecules, parallel to one another, the difficulties would be multiplied considerably. The present discussion assumes that each chromatid consists of a single molecule of DNA.

Recent theories of crossing over (Whitehouse 1963; Holliday 1964; Meselson 1964), varied as they are in detail, rely on the two-stranded character of DNA, with its orderly base pairing, to secure precision at the molecular level. The necessary precision in breakage and reunion, with a tolerance less than 3·4 Å, can be provided by having breakage of single chains of nucleotides in two homologous DNA molecules, followed by melting apart of sister chains and the pairing of non-sister chains of DNA by complementary base pairing. Details of the differences between the particular mechanisms proposed by different authors are irrelevant. All agree in assuming the formation of segments of hybrid DNA. This structure is shown in Figures 3(*b*), 3(*c*), and 3(*d*). The hybrid sections are assumed to be formed by two separate events of breakage and alteration of pairing. In one type [Figs. 3(*b*)(ii), 3(*c*)(ii), and 3(*d*)(ii)] the two sets of breaks result in a recombination for genes in the pure regions on the flanks of the hybrid section. In the other type [Figs. 3(*b*)(i), 3(*c*)(i), and 3(*d*)(i)] there is no recombination in respect of the pure regions.

Thus newly recombinant DNA molecules may be expected to show hybrid sections. They may also show gaps and redundancies, if pairs of breaks are not at exactly corresponding positions in the homologous DNA molecules. Monitoring and repair mechanisms are assumed to detect and correct the various defects, represented as gaps, redundancies, and especially mispaired bases in hybrid sections. Correction of mispaired bases could go either way, restoring the mutant status or producing the normal. Correction is likely to be biased, i.e. the different possible corrections at each site where there are mispaired bases may occur with unequal frequencies.

Allelic recombination, or conversion, is most probably a consequence of formation of segments of hybrid DNA and of correction occurring in them. The relative frequencies of the various lengths of hybrid DNA, the different possible directions of correction, their relative frequencies, and the occurrence of other events in the near neighbourhood of the hybrid segment would be reflected in the statistics associated with allelic recombination, including how flanking markers situated in neighbouring non-hybrid sections are distributed amongst recombinants.

The monitoring and repair mechanisms, as well as the breakage and rejoin processes, all of which involve chemical changes, imply a considerable repertoire of enzymes, each determined by a gene and subject to genetic mechanisms of regulation. Thus it should be possible to find genetic variation in all of these processes, so opening the way to more precise analysis. It is proposed that the observed variations, in the representation of flanking markers amongst the prototrophs arising from allelic recombination, should be studied from this point of view. Already, Smith (1966) has

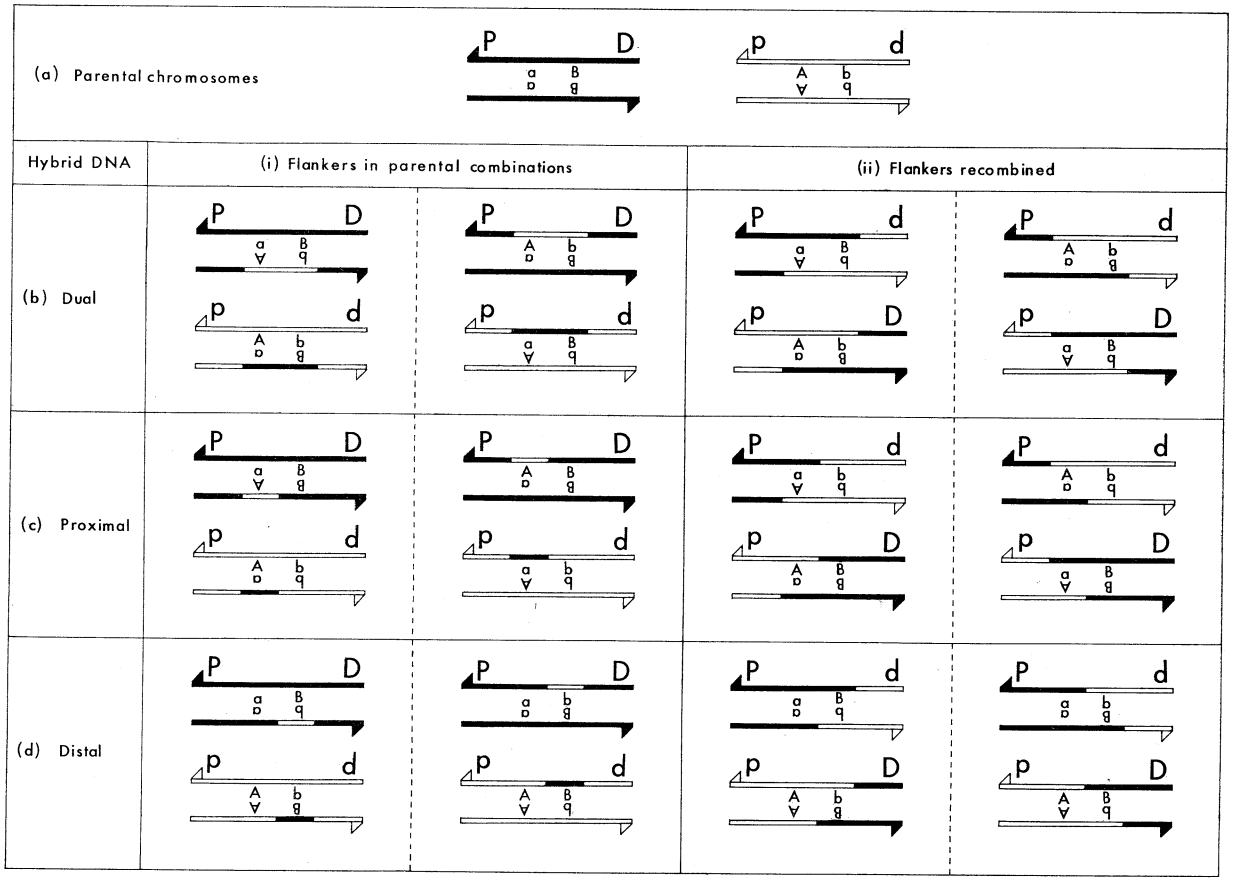

Fig. 3.—Diagrams to show different extents of hybrid DNA in an allelic cross ($m^1 \times m^2$) with flanking markers (P/p, D/d). Mutant bases are shown by lower case letters (a and b), normal by capitals (A and B). The homologous regions of parents are shown in (a); (b), (c), and (d) show them after exchange to give hybrid sections. In (b)(i), (c)(i), and (d)(i) there is no recombination of flanking markers; in (b)(ii), (c)(ii), and (d)(ii) recombination of flankers has occurred. Correction of mispaired bases may restore the mutant status, give double mutants or the wild type. See text for quantitative consequences.

shown in *Neurospora crassa* that variation in representation of the proximal flanker (*pyr–3*, 1298) amongst *his–5*$^+$ prototrophs formed by allelic recombination is due to an unlinked gene, *rec–2*, whose only known effect is on nonallelic recombination in the vicinity of *his–5*.

The manner in which allelic recombination, by correction of mispaired bases in sections of hybrid DNA, may affect the occurrence of flanking markers is seen by reference to the diagrams in Figure 3. The flanking markers are designated by the symbols (*P* and *p*, *D* and *d*) used previously, while the allelic differences, which may give rise to prototrophs, are represented by pairs of letters, relatively inverted, to represent paired bases. Thus $\binom{AB}{vq}$ is the representation of the normal, or prototrophic, condition, while $\binom{aB}{vq}$ and $\binom{Ab}{vq}$ are the m^1 and m^2 mutant conditions.

The diagrams are formulated as though breaks and consequent exchanges have occurred in DNA strands of like polarity in homologous sections of two chromosomes. Three basic cases are possible in which (1) both sites of mutant difference are in the section of hybrid DNA, (2) only the proximal site is in the hybrid DNA, (3) only the distal site is in the hybrid DNA. These are referred to as dual, proximal, and distal. The left-hand break leading to a section of hybrid DNA may occur in either strand of the DNA molecule, presumably with equal frequency. The right-hand break, terminating a hybrid section, may occur in the same strand of the DNA or in the other strand, presumably with equal frequency. The former gives rise to parental combinations (*PD* and *pd*) of the flankers, whereas the latter results in recombinant combinations (*Pd* and *pD*).

At each site of allelic difference, two kinds of mispaired bases are produced in the hybrid DNA. Thus at the m^1 site, there may be $\binom{A}{v}$ or $\binom{a}{v}$. For expression as prototrophs, the hybrid base pairs must be corrected to $\binom{A}{v}$. Similarly, the hybrid pairs $\binom{B}{q}$ and $\binom{b}{q}$ must be corrected to $\binom{B}{q}$. The chance of correction to prototrophy must be assumed to be different for each of the mispaired bases.

If segments of hybridity were dual, covering both of the allelic differences [Figs. 3(*b*)(i) and 3(*b*)(ii)], the two combinations of mispaired bases, namely $\binom{aB}{vq}$ and $\binom{Ab}{vq}$, would occur equally frequently in each of the combinations of flanking markers. Hence, the four combinations of flanking markers occur with equal frequency amongst prototrophs, i.e. $\frac{1}{4}PD$, $\frac{1}{4}pd$, $\frac{1}{4}Pd$, $\frac{1}{4}pD$, undisturbed by any differences between probabilities of correction of the various mispaired bases.

If segments of hybridity were proximal, covering only the m^1 site, only *PD* and *pD* types, which are equally frequent, could give rise, by repair mechanisms, to prototrophs. Since the two kinds of mispaired bases, $\binom{A}{v}$ and $\binom{a}{v}$, occur in both with equal frequency, differences in probability of correction do not affect the representation of prototrophs amongst the combinations of flanking markers. However, combinations of flankers, other than *PD* and *pd*, could arise by crossing over between the distal mutant site and the distal flankers in a proportion *s* of the prototrophs. This would give $\frac{1}{2}(1-s)PD$, $\frac{1}{2}s.pd$, $\frac{1}{2}s.Pd$, and $\frac{1}{2}(1-s)pD$ amongst the prototrophs, a distribution in which the proximal flankers are equally frequent and the distal flankers very unequal with a ratio of $(1-s)D$ to $s.d$. Crossing over between the proximal flankers and the proximal allelic difference does not enter into the expectations, simply because *P* and *p* are equal in frequency.

Similar arguments apply to the cases in which the segments of hybridity are distal, covering only the m^2 site. The expected proportions amongst the prototrophs are $\frac{1}{2}r.PD$, $\frac{1}{2}(1-r)pd$, $\frac{1}{2}r.Pd$, and $\frac{1}{2}(1-r)pD$ where r is the frequency of crossing over between P/p and $m^1/+$.

A remarkable feature of this analysis is the lack of effect of different probabilities of correction to prototrophy of the different possible couples of mispaired bases. This extends to the situation in which mispaired bases are not corrected, but instead separate at the next replication, to give a normal and mutant site in the respective daughters. This is the effect which is thought to give rise to 5 : 3 and other classes of abnormal asci. Although factors of this kind do not affect the relative representation of different combinations of flanking markers amongst the prototrophs, they could affect the frequency of prototrophs to at least a moderate extent.

With hybridity restricted to regions containing only one allelic difference, the frequency of *Pd* would be relatively low. This expectation is approximated by the data (Table 1) on the *pab* locus of *Aspergillus* and the *wx* locus of maize. The relatively low frequency of both *PD* and *Pd* shown by the data on *his–5* of *Neurospora* (Table 1) would be approximated by a situation in which hybridity was restricted to the distal allelic difference.

The equality expected with dual segments of hybrid DNA is approximated by much of the data encountered in study of the *his–1* locus in *Neurospora* (Table 1), except that *Pd* is commonly less frequent than the other three types. This could be accounted for if sometimes the segment of hybridity extended over only one site of allelic difference. Formulae could be developed to evaluate the relative contributions derived from the different extents of hybrid DNA.

The part of the *his–1* data (Table 1, fifth entry) in which both *PD* and *Pd* are relatively reduced, could be explained by a greater preponderance of hybrid segments covering only the distal allele difference. Other *his–1* data (Table 1, sixth entry) seem to show a reversal in the order of the two *his–1* alleles together with a preponderance of hybrid sections covering what would be the distal allele difference.

It will be noticed that no factor so far considered leads to the kind of conflict between the orders deduced from rules II and III shown in Murray's (1963) data on *me–2*. Hence it appears that the theory, as formulated at present, is defective.

VI. References

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