THE DETECTION OF LINKAGE IN TETRAD ANALYSIS

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ESTABLISHING the existence of linkage between genes at two loci is a distinct problem from estimating map-distance once genes are known to be linked. This paper considers two questions that concern linkage detection in organisms with genetically analyzable tetrads. First, what criterion is best for establishing linkage when tetrad segregations are analyzed? And second, what are the advantages of tetrads compared to random single strands for indicating linkage?

CHOICE OF A VALID AND EFFICIENT CRITERION FOR LINKAGE DETECTION WITH TETRADS

In classical single-strand analysis, linkage between two genes is indicated when the ratio of recombination to non-recombination gametes is significantly less than equality. For organisms (chiefly lower plants) where all four products of a single meiosis can be recovered, similar criteria have been adopted for detecting linkage. Ratios of whole tetrads have rarely been used, but segregants collected as tetrads have instead been treated as populations of single strands (e.g., POMPER and BURKHOLDER 1949) or of half tetrads (e.g., WHITEHOUSE 1942) for purposes of determining if recombination : non-recombination numbers differ significantly from the 1 : 1 ratio expected with independent segregation. (By half tetrad is meant specifically a pair of complementary products, $AB + ab$ or $Ab + aB$, originating from the same tetrad.) When segregants that have been obtained as tetrads are treated in this way, two types of error may result. Linkage may either be indicated spuriously where none exists, or linkage may remain concealed that would become apparent if the data were treated more critically.

A dihybrid zygote ($AaBb$), from gametes AB and ab, can produce any of three possible tetrad types: parental ditype ($AB + AB + ab + ab$), non-parental ditype ($Ab + Ab + aB + aB$) and tetratype ($AB + Ab + aB + ab$). Observed numbers of these classes are the basic data of tetrad analysis. Parental ditype (PD) and non-parental ditype (NPD) tetrads are equally probable if genes are unlinked, and it will be shown that a significant departure from equality of these two classes is both a reliable and an efficient criterion for establishing linkage when tetrads are used. The advantages of ditype tetrad numbers will be discussed in connection with examples in table 1, where alternative methods are used to examine data from a variety of tetrad segregations.

Non-independence of component parts of tetrads in testing departures from random segregation. It is not correct to assume that the two half tetrads from a single meiotic segregation are independent with respect to their recombinant

TABLE 1

The significance of deviations from random segregation indicated by ratios of non-parental: parental ditype tetrads and of recombinant: parental half-tetrads.

Example	Observed tetrad numbers, PD:NPD:T	Half-tetrad numbers, R:P	Probability* that specified numerical ratio is a random deviation from 1:1			% recombination	Organism	Segregating characters	Reference
			Tetrad numbers, NPD:PD	Half-tetrad numbers, R:P	Half-tetrad numbers × 1/2, R:P				
1	2	3	4	5	6	7	8	9	10
1	40:30: 0	60:80	.14	.05	.14	43**	*Sphaerocarpus donnellii*	v, sex	Knapp 1936
2	51:45: 0	90:102	.30	.21	.30	47**	"	d, sex	" "
3	25:20: 0	40:50	.28	.17	.28	44**	*Neurospora tetrasperma*	d, mt	Dodge et al. 1945
4	11: 5: 1	11:23	.10	.03	.11	32**	Marchantia	fa, hy	Burgeff 1943
5	11: 3: 2	8:24	.03	<.01	.04	25	*Neurospora crassa*	35203, 35301	Houlahan et al. 1949
6	1: 3: 43	....	....	....	....	52	Sphaerocarpus	u, sex	Knapp 1936
7	50:59:413	....	....	....	....	51	"	c, sex	" "
8	44:28:119	175:207	.04	.06	.14	46	*Saccharomyces cerevisiae*	g, me	Lindegren 1949
9	14: 4: 43	51:71	.02	.04	.13	42	"	ma, pb	" "
10	17: 5: 45	55:79	<.01	.02	.09	41	"	pb, alpha	" "
11	34:17:110	144:178	.01	.03	.10	45	Glomerella	conidial A, B	Chilton et al. 1949
12	10: 2: 20	24:40	.02	.03	.11	38	*Dunaliella salina*	r, mt	Lerche 1937
13	9: 2: 15	19:33	.03	.04	.12	37	*Chlamydomonas Moewusii*	b, mt	Lewin 1950
14	8: 0: 11	11:27	.004	.007	.05	29	"	t, 1	" "
15	22: 6: 48	60:92	.002	.006	.04	39	*Neurospora crassa*	C94, C141	Haas et al. 1952
16	24: 7: 63	77:111	.002	.008	.05	41	"	C141, T1710	" "

* P-values (one-sided) were obtained using a table of normal deviates and applying Yates's correction for continuity (McMullen 1936), except for small values in examples 14–16, which are from Warwick's tables.

** Location in separate linkage groups is indicated by low T and high NPD frequencies.

or parental composition, either for linked or unlinked genes. To use actual numbers of half tetrads may therefore be to increase the population size arbitrarily above its proper value, with the result that excessive significance is attributed to deviations. This is a serious objection to using component tetrad parts for linkage detection.

The null hypothesis, independent segregation, predicts equal numbers of parental and non-parental ditype tetrads, but sets no limits to the proportion of tetratypes. Establishment of non-independence thus depends solely upon ditype tetrads, where the constitution of any portion defines the whole tetrad. For efficiency in detecting linkage, comparisons should be limited to ditype segregations. Sister halves from ditypes are identical, and may not be treated as statistically independent units. On the other hand, efficiency might be ignored, and the uninformative tetratypes included for analysis. Here also sister half tetrads may not be considered independent, because in crosses where ditype segregations are in excess over tetratypes a positive correlation exists between sister halves, and where tetratypes predominate, a negative correlation obtains.

The error from using actual numbers of half tetrads is most apparent when tetratype (T) segregations are infrequent or absent. In example 1, table 1, with 40 PD : 30 NPD : 0 T tetrads, the correct population size is 70, not 140, and in 70 segregations of independent genes, the probability of obtaining the observed ratio is clearly that of 40 : 30 occurring when 35 : 35 is expected, not 80 : 60 against an expected 70 : 70.

Observed numbers of parental and recombination single strands can validly be used for testing the null hypothesis of independent segregation where each strand originates from a separate meiosis, a situation approximated in classical methods with higher plants and animals. Half-tetrad numbers would be suitable units for comparison if each represented a separate segregation, as do the two strands recovered in attached-X Drosophila females, Habrobracon impaternate daughters or Bombyx mosaics from binucleate eggs. But where a sample of segregants consists of a series of whole tetrads, raw numbers of half (or quarter) tetrads are not valid for significance tests.

Half-tetrad ratios might perhaps be used if the numbers were first reduced so as not to attribute false significance to deviations. Such an adjustment was made by dividing actual half-tetrad numbers by two before computing the probabilities given in column 6 of table 1. Nothing is gained by computing adjusted half-tetrad ratios in this way, since they are less sensitive for detecting linkage, and more laborious to obtain, than ditype tetrad ratios.

Loss of sensitivity due to including tetratype segregations. A second major objection to using half- or quarter-tetrad numbers for linkage detection is that segregants from tetratype tetrads are then included, and these are irrelevant for establishing linkage. Ditype ratios are able to provide a more efficient and sensitive criterion of non-independence than half-tetrad or quarter-tetrad ratios because irrelevant segregations are excluded.

Unlinked genes may produce tetratypes in any proportion whatever, from zero to 100% of the total population (see WHITEHOUSE 1949, p. 231). The

frequency of tetratypes may be zero if two genes are at centromeres on different chromosomes (examples 1–3, table 1). At the other extreme, all segregations could result in tetratypes if one gene were at a centromere and another, unlinked, gene were separated from its centromere by an interval within which a single exchange always occurred, a condition approached in examples 6 and 7. No evidence for linkage is gained, therefore, by including data from tetratype segregations (as is done if half tetrads or single strands are used from the whole population of tetrads), and inclusion of numerous tetratypes may even conceal a significant departure from randomness that is apparent from the ratio of ditype tetrads (examples 8 ff.).

Failure of tetratype segregations to contribute evidence for linkage does not imply that they are unnecessary for other purposes. Tetratype frequencies are indeed essential for such operations as determining the intensity of linkage once its existence has been established, and it is important that all segregations, including tetratypes, be included when tetrad data are collected and published.

In particularly favorable cases tetratype frequencies may make it possible to establish from two-point segregations that genes are *not* linked, e. g., where the two ditype classes are numerous and equally frequent, but tetratypes are rare (examples 1–4, table 1; WHITEHOUSE 1949, p. 232). A very rigid criterion for non-linkage would be a significant deviation in excess of 1NPD : 2T. This is the maximum ratio attainable for linked genes, barring chromatid interference, but would be realized only in the rare case where all exchanges occurred as doubles. A more practical test would be a significant deviation in excess of 1NPD : 4T, which is the maximum ordinarily expected for genes distantly spaced in the same linkage group.

Application of ditype tetrad ratios to linkage detection. A variety of segregations have been gathered in table 1 and examined for evidence of linkage, using ditype tetrad ratios as a criterion. The results (column 4) stand in contrast to probabilities obtained when half-tetrad numbers are used (column 5). In examples 1–5 deviations of ditype tetrad numbers from 1 : 1 are less significant than those of half tetrads. The low probability values in column 5 are not applicable for linkage detection because sister half tetrads are not independent of one another, and use of these probabilities might lead one to accept linkage with false confidence. If the half-tetrad numbers are divided by two, so as to reduce the population from its inflated size, the new P-values (column 6) agree with those from ditype tetrads.

Examples 8–16 show, in contrast, how linkage may be overlooked if half tetrads rather than ditype tetrads are used as a criterion. (Linkage has in fact not previously been recognized in several of these cases, although ditype ratios indicate that it is highly probable.) The lesser significance of the half-tetrad deviations is due to including data from tetratype tetrads, which contribute parental and non-parental halves in equal numbers.

These applications support the contention that ditype tetrad numbers are generally more reliable and more efficient than half-tetrad numbers for indicating linkage. RIZET and ENGELMANN (1949, pp. 242, 257) have clearly recognized the role of ditype tetrads in linkage detection, and CATCHESIDE (1951, p. 25), following LINDEGREN (1933), has also noted that these two

tetrad classes provide a useful criterion of non-independence, but has unfortunately restricted his comparison to tetrads in which genes at both loci have segregated at the first meiotic division.

In practice, ditype numbers are extremely simple and convenient to use for determining the probability of linkage. Table 2 lists the lowest numerical ratios that deviate in one direction from 1 : 1 sufficiently to attain each of three confidence levels. These are the smallest PD : NPD ratios capable of providing

TABLE 2

Smallest numerical ratios showing significant deviation in one direction from 1 : 1.

Total numbers	Ratios attaining significance level (one-sided)			Total numbers	Ratios attaining significance level (one-sided)		
	5%	2 1/2%	1%		5%	2 1/2%	1%
5	5:0	....	....	28	19:9	20:8	21:7
6	6:0	6:0	....	29	20:9	21:8	22:7
7	7:0	7:0	7:0	30	20:10	21:9	22:8
8	7:1	8:0	8:0	31	21:10	22:9	23:8
9	8:1	8:1	9:0	32	22:10	22:10	23:9
10	9:1	9:1	10:0	33	22:11	23:10	24:9
11	9:2	10:1	10:1	34	23:11	24:10	25:9
12	10:2	10:2	11:1	35	23:12	24:11	25:10
13	10:3	11:2	12:1	36	24:12	25:11	26:10
14	11:3	12:2	12:2	37	24:13	25:12	26:11
15	12:3	12:3	13:2	38	25:13	26:12	27:11
16	12:4	13:3	14:2	39	26:13	27:12	28:11
17	13:4	13:4	14:3	40	26:14	27:13	28:12
18	13:5	14:4	15:3	41	27:14	28:13	29:12
19	14:5	15:4	15:4	42	27:15	28:14	29:13
20	15:5	15:5	16:4	43	28:15	29:14	30:13
21	15:6	16:5	17:4	44	28:16	29:15	31:13
22	16:6	17:5	17:5	45	29:16	30:15	31:14
23	16:7	17:6	18:5	46	30:16	31:15	32:14
24	17:7	18:6	19:5	47	30:17	31:16	32:15
25	18:7	18:7	19:6	48	31:17	32:16	33:15
26	18:8	19:7	20:6	49	31:18	32:17	34:15
27	19:8	20:7	20:7	50	32:18	33:17	34:16

significant indications of linkage. The values in table 2, obtained by using WARWICK's (1932) tables, can be determined with equal accuracy from binomial probability paper (MOSTELLER and TUKEY 1949), which is also useful for handling numbers beyond 50.

UTILITY OF RANDOM SINGLE STRANDS VERSUS TETRADS FOR ESTABLISHING LINKAGE

We have till now been concerned with the single problem, how best to detect linkage when segregants have been collected as tetrads. A second, distinct question can now be examined. Are tetrads preferable to random isolates for revealing linkage? Comparative efficiencies and reliabilities will be important for the choice, as will the prospect of obtaining other types of information from the same data used to establish linkage.

TABLE 3

Smallest numbers of tetrads and of random single strands likely to indicate linkage across intervals having specified exchange distributions.

Example no.*	Exchanges distributed among bivalents as for:	Length of interval**	Segregation probabilities	Smallest numbers likely to provide significant evidence of linkage***				Relative efficiency. randoms: tetrads
				Random strands		Ditype tetrads		
			PD : NPD : T	P + R	Total	PD + NPD	Total	Total tetrads
1	2	3	4	5		6		7	8
1	Alleles	0	1 : 0 : 0	5 + 0	5	5 + 0	5	5	4.0
2	Zero interference	25	0.628 : 0.021 : 0.352	7 + 1	8	5 + 0	5	8	4.0
3	" "	50	0.425 : 0.057 : 0.517	15 + 6	21	5 + 0	5	10	1.9
4	" "	100	0.251 : 0.116 : 0.633	89 + 67	156	16 + 7	23	63	1.6
5	" "	125	0.216 : 0.134 : 0.650	218 + 184	402	35 + 21	56	161	1.6
6	Complete interference	12.5	0.75 : 0 : 0.25	5 + 0	5	5 + 0	5	7	5.6
7	" "	25.0	0.50 : 0 : 0.50	11 + 3	14	5 + 0	5	10	2.9
8	" "	37.5	0.25 : 0 : 0.75	28 + 16	44	5 + 0	5	20	1.8
9	" "	45.0	0.10 : 0 : 0.90	154 + 125	279	5 + 0	5	50	0.7
10	Saccharomyces, *pn-in*	26	0.488 : 0.000 : 0.512	10 + 3	13	5 + 0	5	10	3.1
11	" , *g-me*	>46	0.230 : 0.147 : 0.623	219 + 185	404	44 + 28	72	192	1.9
12	Glomerella, Con.A-B	>45	0.211 : 0.106 : 0.683	143 + 115	258	23 + 11	34	108	1.7
13	Chlamydomonas, *b-mt*	>37	0.346 : 0.077 : 0.576	30 + 17	47	7 + 1	8	19	1.6
14	Schizophyllum, *A-s*	>27	0.500 : 0.045 : 0.455	12 + 4	16	5 + 0	5	9	2.2
15	Marchantia, *he-ac*	>27	0.474 : 0.013 : 0.513	10 + 3	13	5 + 0	5	10	3.1
16	Neurospora, *c-mt*	12	0.765 : 0.009 : 0.226	5 + 0	5	5 + 0	5	6	4.8
17	Zea, lg_1-v_4.	>60	0.182 : 0.054 : 0.764	97 + 74	171	9 + 2	11	47	1.1
18	Drosophila, *sc-f*	62	0.141 : 0.072 : 0.787	314 + 273	587	19 + 9	28	133	0.9
19	" , *y-bb*	>77	0.173 : 0.117 : 0.710	460 + 410	870	47 + 31	78	270	1.2
20	*Allium macranthum*, chiasmata, short chromosomes	70.4	0.140 : 0.109 : 0.750	1418 + 1330	2748	101 + 78	179	717	1.0
21	Chorthippus, chiasmata, short chromosome	52.0	0.010 : 0.010 : 0.980	....	....	....	....	....	....

*Sources of data: Ex. 10, 11, Lindegren 1949. Ex. 12, Chilton et al. 1949. Ex. 13, Lewin 1950. Ex. 14, Papazian 1951. Ex. 15, Burgeff 1943. Ex. 16, Mather et al. 1942. Ex. 17, Rhoades 1933. Ex. 18, Bridges et al. 1926. Ex. 19, Reck 1936. Ex. 20, Levan 1933. Ex. 21, Darlington 1932. Segregation probabilities in 17–19 are based on exchange frequencies calculated from genetic data by Ludwig (1938, p. 142).

**Values in map-units are from the experiments cited.

***$P \leq 0.05$, one-sided, for deviation from 1 : 1, and $P \geq 0.50$ for attaining or exceeding these proportions, on the basis of the ratios calculated from column 4. See appendix regarding computations.

Comparative efficiencies for linkage detection. Tetrads are less efficient than random strands for estimating linkage intensity (MATHER and BEALE 1942; PAPAZIAN 1952), but efficiencies of the two for *detecting* linkage have evidently never been compared. Table 3 gives the smallest numbers of tetrads and of random single strands likely to reveal linkage over various distances, for hypothetical models with complete interference (examples 6–9) and with none (2–5), and for cases where the frequencies of segregation types are known from genetic (10–19) or from cytological (20, 21) data. (Calculations for table 3 are described in an appendix.) These examples indicate that linkage can ordinarily be detected more efficiently with random single strands than with tetrads, i.e., that the amount of information per strand is greater for strands collected singly.

Three factors appear to be important in determining the relative efficiencies of tetrads compared to random isolates. (a) *The non-independence of constituent parts of tetrads.* About four times as many strands must usually be examined when tetrads are analyzed as when strands are collected at random, in order to reveal linkage over short distances (table 3, examples 1, 2, 6, 16). (b) *The interval length between markers.* Information per strand decreases from a maximum at short distances to zero for long intervals, both for random isolates and tetrads. The advantage of random strands over tetrads accordingly diminishes with distance (examples 2–5, 6–9, 10–11) until linkage can no longer be detected by either method across an interval where one or more exchanges occur in every bivalent (example 21). (c) *The intensity and pattern of interference.* Efficiencies of the two methods may be affected differentially by interference, so that tetrads can sometimes achieve or exceed the efficiency of random strands in the case of long intervals having a predominance of tetratype segregations (examples 9, 18).

In practice, the choice between tetrads and random strands will not depend solely on theoretical efficiencies (information per strand), but also on how laborious it is to collect strands by the two methods. In most organisms (liverworts are a possible exception) less work is required to obtain four strands at random than to isolate the four strands that compose a tetrad. The superior theoretical efficiency of random isolates relative to tetrads may thus be amplified by the greater ease with which random products can be obtained experimentally.

Sources of error in linkage detection. Differential viability or false identification could lead to serious errors either with tetrads or with single strands. Selection may operate either between individual segregants or between tetrads. While tetrad analysis sometimes makes it possible to identify a missing product by inference, and thus to decrease errors due to differential survival of particular segregant types, selection may also operate *among* tetrads against particular segregation classes that fail to produce complete complements of progeny. Such inter-tetrad selection might result, for example, from rejecting Neurospora asci having fewer than eight, or fewer than six, normal spores, and

could produce PD: NPD tetrad ratios that were more seriously in error than random-strand ratios from the same cross.

Whole tetrads seem less likely to be misclassified than single strands, but misidentification (or irregular segregation) could distort tetrad ratios if the constitution of incomplete tetrads is deduced by filling in missing products.

Other features of tetrads that may influence a choice of methods. Since random strands are usually more efficient than tetrads for linkage detection, and are perhaps no more subject to systematic errors, they would probably be preferred to tetrads if establishment of linkage were an isolated aim. But considerations other than accuracy and efficiency for detecting linkage may influence the choice.

Tetrads possess a number of advantages over random strands. Tetrad analysis is important for studying chromatid interference and for mapping centromeres (LINDEGREN 1933; WHITEHOUSE 1942; PAPAZIAN 1952). If a marker is available that regularly segregates with the centromere on one chromosome, the position of the centromere on any other chromosome can be mapped, even with unordered tetrads (e.g., KNAPP 1936). Efficiency is gained in mapping when the centromere distance of a gene is known so that tests can be made for linkage with specific markers that have similar centromere distances.

Tetrad analysis may indicate that particular variants result from crossing-over within a compound locus rather than from point mutation (PAPAZIAN 1951), even though the locus is not bridged with markers. (This is the simplest hypothesis where two reciprocally different new "alleles" appear as members of one tetrad.)

Tetrads show directly that crossing-over occurs between chromatids at the four-strand stage of meiosis, and segregation ratios in tetrads, being absolute rather than statistical, provide the most direct possible demonstration of the Mendelian basis of an inherited difference, as was recently pointed out by Quintanilha (cited in GUSTAFSSON 1951). Conversely, tetrads furnish direct evidence of extrachromosomal inheritance (CHEN et al. 1950). Aberrations or lethal mutations may be revealed that are undetectable with single strands (KNAPP 1937). Tetrads also make it possible to correct map-distance estimates for the occurrence of those double crossovers that do not result in recombination of the markers bounding an interval (PERKINS 1949).

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SUMMARY

When complete tetrads are analyzed, the most satisfactory criterion of departures from random segregation is a significant deviation from equality

of parental and non-parental ditype tetrads. Treatment of tetrad data as though the component half tetrads or single strands were independent is not valid for purposes of linkage detection, and may either obscure the existence of linkage, or indicate linkage spuriously where none obtains.

Linkage can ordinarily be detected more efficiently with random single strands than with tetrads. Nevertheless, tetrads may sometimes be preferred because of their superiority for purposes other than linkage detection.

APPENDIX: CALCULATIONS FOR TABLE 3

Distribution of exchanges among bivalents. Relative proportions of PD, NPD and T segregations (column 4) are obtained directly only when tetrads are analyzed (examples 10–16), but can be computed if the proportions are known of bivalents having different numbers of exchanges, i. e., bivalents of different rank (WEINSTEIN 1936). Relative frequencies of different ranks can be estimated from chiasma counts (examples 20, 21), or from multiple-point single-strand crossover data (MATHER 1933; WEINSTEIN 1936; LUDWIG 1938; examples 17–19). They are also known for complete interference (examples 6–9) where all exchanges occur as singles, and for zero interference (examples 2–5) where exchanges would be distributed among the bivalents at random, and probabilities for occurrence of bivalents of increasing rank are given by the successive terms of a Poisson series.

Calculation of tetrad proportions if the distribution of exchange frequencies is known. The probability t_r that a bivalent of rank r will segregate as a tetratype can be obtained from chromosome diagrams, or by use of the progression $t_r = (2/3)\ [1 - (-1/2)^r]$ adapted from Mather (1935). For $r > 0$, each ditype has a probability of occurrence $d_r = (1 - t_r)/2$, but for linked genes, all segregations are parental ditypes when $r = 0$. If one knows the probabilities of occurrence of bivalents of different rank, P_r, these equations give the corresponding probabilities of obtaining each of the 3 segregation types—PD, NPD or T—so that appropriate probabilities for individual ranks can be multiplied, and contributions from bivalents of all ranks then summed, to obtain overall probabilities for occurrence of each tetrad type in a specified cross. The probability of tetratypes, $t = \sum_{r=0}^{\infty} P_r t_r$, the probability of NPD tetrads $= d = \sum_{r=1}^{\infty} P_r d_r$, and the probability of PD tetrads $= d + P_o$. Column 4 values in examples 2–9 and 17–21 were obtained in this way.

Smallest numbers of random strands likely to indicate linkage (Column 5). The probabilities of obtaining parental strands from PD, NPD and T segregations are 1, 0 and 0.5 respectively, whence ratios of parental : recombinant strands can be calculated from tetrad ratios. It is desired to obtain, for column 5, the smallest number of random isolates expected to indicate linkage in at least 50% of tests. The smallest R : P numbers likely ($P \geq 0.5$ for attainment if segregations are as in column 4) to show a significant deviation from 1 : 1 ($P \leq 0.05$, one-sided) can readily be determined graphically, using MOSTELLER and TUKEY'S (1949) binomial probability paper, and determining the paired count whose apex falls between and beyond the intersection of the R : P split and a line paralleling the 50 : 50 split at 8.4 mm distance.

Smallest numbers of ditype tetrads likely to indicate linkage (Column 6). NPD : PD ratios from column 4 are used directly to plot a split on binomial probability paper, and the smallest paired count is determined as described above.

Smallest total number of tetrads required (Column 7). Once minimum ditype numbers have been specified, the total number of tetrads that must be analyzed to provide enough ditypes depends in turn upon the tetratype proportions. Again, binomial probability paper can be used, plotting the tetratype : ditype split and determining the paired

count nearest the point where this split intersects a horizonal line passing through the minimum ditype number.

Relative efficiencies (Column 8). Since information per strand is inversely proportional to the number of strands required, the relative efficiency of *random strands : tetrads* is expressed as the proportion: (*total strands required as tetrads*)/(*total strands required as randoms*). Graphical and arithmetic errors are small compared to the differences in efficiency.

The unexpected appearance of relative efficiencies exceeding four (examples 6, 16) is due to the fact that calculations are based on the smallest numbers of random strands and of tetrads that are likely ($P \geq 0.5$) to provide significant evidence of linkage. Relative efficiencies would attain but not exceed four if the comparison had been based on the *mean* numbers required to reveal linkage, rather than on the smallest numbers *likely* to do so. In general, results from the two measures of efficiency would differ but little. Conclusions drawn from table 3 would be the same whichever criterion was used.

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