Genet. Res., Camb. (1962), **3**, *pp.* 196-209
With 1 *text figure*
Printed in Great Britain

Preferential segregation in *Saccharomyces**

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(*Received* 18 *August* 1961)

1. INTRODUCTION

Recombination values of less than 50% between genes located on different chromosomes have been observed and given the names 'false linkage' (Longley, 1945), and 'quasi-linkage' (Michie, 1953; Wallace, 1953) to distinguish them from ordinary linkage between genes on the same chromosome. This phenomenon appears to be due to the action of specific sites (termed variously as 'sites of affinity' and 'neocentromeres') situated on non-homologous chromosomes, which segregate preferentially to the same meiotic pole. Longley demonstrated cytologically that certain knobbed chromosomes of maize segregated preferentially with an abnormal chromosome 10.

In lieu of cytological evidence, preferential segregation, if it occurs, must be demonstrated on the basis of genetic analyses alone. In terms of single-strand data, preferential segregation would be indicated in two ways: (1) the occurrence of more than 50% recombination (to an extent not explicable by positive chromatid interference) and (2) contradictions in linear gene order resulting from quasi-linkage. Of course, less than 50% recombination between genes known to lie on different chromosomes would indicate preferential segregation, but such a deduction requires some independent method for determining that two genes are in fact on different chromosomes. Independence data would not be enough to ensure this, since, taken together with data alleged to indicate preferential segregation, it only indicates heterogeneity of the recombination frequencies. In this case one would have to rule out the possibility that heterogeneity in recombination frequency may involve distantly-linked genes segregating in stocks carrying inversions and other non-homologies. However, in many instances, there *are* methods for showing that gene exhibiting less than 50% recombination lie on different chromosomes. As examples, we have (1) the establishment of cytological correlates (as in *Zea mays*, Longley, 1945), (2) the use of trisomics (genes *j* and *wt* in tomatoes, Rick & Barton, 1954), and, (3) in tetrad analysis, the location of centromeres (in *Saccharomyces* genes α and ga_1, Shult & Desborough, 1960; and genes p and tr_1, Hawthorne & Mortimer, 1960).

2. GREATER THAN 50% RECOMBINATION

Instances of recombination frequencies significantly in excess of 50% have been recorded by a number of workers (Table 1). In tetrad data, more than 50% re-

* This work has been supported by the American Cancer Society and U.S.P.H.S. C–4682.

combination occurs only when the number of non-parental ditype tetrads (NPD) exceeds the number of parental ditype tetrads (PD). Such tetrad distributions are designated R-distributions and are by no means rare events. (For experimental procedures and methods of genetic analysis involving tetrad data see Desborough & Lindegren (1959) and Shult & Desborough (1960).) In data from the Carbondale yeast stocks, a total of 1487 tetrad distributions (one tetrad distribution for each pair of different gene-markers in each family) have been recorded for Families 80 to 116. Of these, 32 distributions exhibit an excess of non-parental ditype tetrads over parental ditype tetrads to a degree which is significant at the 1% level. A list of

Table 1. *Instances of high recombination frequencies*

Author	Genes	Frequency of recombination	Organism
Wright, M. E. (1947)	sex and 'shaker' (sh_2)	56·73% $p = 0{\cdot}42\%$	*Mus musculus*
	sex and 'wavy' (wv_2)	56·07% $p = 0{\cdot}94\%$	
Hawthorne (1955)	uracil and methionine	57·75% $p = 3{\cdot}3\%$	*Saccharomyces*
	histidine and methionine	60·56% $p = 2{\cdot}8\%$	
Shult, E. E. & Lindegren, C. C. (1958)	inositol and proline	73·77% $p < 10^{-5}$	*Saccharomyces*
	inositol and lysine	62·29% $p < 10^{-3}$	
	adenine and proline	63·28% $p < 10^{-3}$	
Wallace (Wright), M. E. (1958)	dominant pied (*W*) and fidget (*fi*)	63·70% $\chi^2 = 6{\cdot}87$	*Mus musculus*
	W and Danforth's short tail (*sd*)	59·40% $\chi^2 = 3{\cdot}18$	

these distribution with the pair of markers involved and the family in which it occurred, is presented in Table 2. Table 3 shows the number of tetrads dissected, the percentage spore germination and the number of irregular tetrads for each family. Of course, a certain number of the R-distributions presented in Table 2 will occur by chance. At the 1% level, the number of significant distributions which would be expected in 1487 distributions is $0{\cdot}5\% \times 1487 = 7{\cdot}435$. This number departs radically from 32, the number observed. Assuming there is no mechanism for producing the excess of NPD over PD tetrads other than random sampling itself, and that any one distribution is independent of its counterpart in any other family and of distributions between other pairs of markers, the observed number of R-distributions significantly exceeds the expected number, with z (the normal curve parameter) equal to 9·0312 (p is of the order of 10^{-6}). However, since some of these genes

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are linked the tetrad distributions listed cannot be considered independent. Thus, the five R-distributions involving cu_2 in Family 108 may be counted as one 'accident', rather than five, since ur_3, *ch*, hi_1, is_1 and *th* exhibit direct linkage with each other. Similarly, the two R-distributions for α–*an* and α–is_1 in Family 107 may be

Table 2. *Tetrad distributions exhibiting greater than* 50% *recombination*

		Tetrad distribution		
Pair of markers	Family	PD	NPD	T
α–*an*	107	5	16	24
α–*ch*	111	2	25	34
α–cu_2	99	3	12	15
α–ga_1	107	7	22	18
α–is_1	107	3	14	31
α–*pr*	108	7	20	59
ac_1–ad_1	96	0	13	27
ac_1–ad_1	87	3	12	2
ac_1–*cd*	110	1	15	8
ac_1–*ch*	97	1	8	9
ac_1–*ch*	109	2	11	35
ac_1–*th*	97	1	9	7
ad_1–*th*	96	2	14	27
ad_1–ur_3	91	10	26	7
ch–cu_2	108	6	48	56
ch–*my*	98	1	8	34
cu_2–hi_1	108	11	49	73
cu_2–is_1	108	12	35	84
cu_2–*th*	108	10	37	40
cu_2–ur_3	108	12	35	88
is_1–*ma*	91	0	8	14
ly_3–*me*	90	1	9	32
na–*ch*	116	14	33	72
na–*ca*	116	12	31	75
mg_1–*pa*	90	1	8	16
mg_1–*tu*	98	2	12	36
pa–*pr*	93	10	24	58
pa–*th*	98	3	16	23
ph–*pr*	88	7	24	46
pr–*th*	97	1	10	13
pr–*th*	111	7	24	47

considered as one instance of an R-distribution since *an* and is_1 are linked on the same chromosome (they recombine in about 12% of the meioses). The two R-distributions for *na*–*ca* and *na*–*ch* in Family 116, may also be taken to represent *one* event since *ca* and *ch* are very closely linked (they recombine in less than 2% of the meioses). Finally, two instances in Family 97, that for ac_1–*ch* and that for ac_1–*th* may be counted as one (although, as will be seen later, ordinary concepts of chromosomal linkage do not account readily for the appearance of linkage between *ch* and *th*).

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Table 3. *The total number of tetrads dissected for each family, the percentage spore germination, and the irregular or untested tetrads for genes within a family*

Family	No. of asci dissected	Percentage spore germination	Gene	Irregular tetrads	Tetrads not tested
Family 87	25	99·0	ac_1	6	1
			ad_1	1	1
Family 88	107	90·7	*ph*	22	1
			pr	30	1
Family 90	53	94·3	ly_3	2	8
			me	1	1
			mg_1	3	3
			pa	3	16
Family 91	76	94·7	ad_1	2	5
			ur_3	2	5
			is_1	2	5
			ma	1	1
Family 93	103	92·2	*pa*	4	2
			pr	2	2
Family 96	59	92·8	ac_1	15	1
			ad_1	5	0
			th	15	0
Family 97	30	81·7	ac_1	2	9
			ch	3	3
			th	3	3
			pr	1	2
Family 98	50	91·2	*ch*	4	0
			my	2	0
			mg_1	0	1
			tu	0	1
Family 99	32	97·7	α	0	1
			cu_2	1	1
Family 107	56	79·9	α	0	3
			an	2	5
			ga_1	1	3
			is_1	1	7
Family 108	194	93·6	*th*	57	6
			ur_3	6	6
			hi_1	14	2
			is_1	15	3
			an	13	2
			ch	34	5
			cu_2	6	6
Family 109	64	88·7	ac_1	0	26
			ch	2	0
Family 110	38	91·4	ac_1	0	8
			cd	0	1

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Table 3—*continued*

Family	No. of asci dissected	Percentage spore germination	Gene	Irregular tetrads	Tetrads not tested
Family 111	102	94·9	*α*	0	17
			ch	5	0
			pr	4	0
			th	24	0
Family 116	142	95·8	*na*	24	1
			ch	2	1
			ca	3	1

Condensing in this way, the total number of R-distributions (this time, independent) is now 25, a figure which still differs enormously from 7·435 (p is well less than 5×10^{-5}). The fact that there are too many R-distributions suggests the presence of some mechanism which would account for them.

Of the genes listed here, *α*, ur_3, ga_1, ad_1, *ch*, cu_2, *th*, *pr*, *ph*, *my* and ac_1 are known to be centromere markers (Lindegren *et al.* (1959), Desborough & Lindegren (1959), Hawthorne & Mortimer (1960)). Reference to Table 2 readily shows that the majority of instances of reverse linkage *do* involve markers linked to the centromeres of non-homologous chromosomes. In fact the only exceptions to this rule are *α–an* and *α–*is_1 in Family 107, ac_1*–cd* in Family 110, is_1*–ma* in Family 91, ly_3*–me* in Family 90, *mg–pa* in Family 90, and *mg–tu* in Family 98. Although the genes *cd*, *ma* and *me* have been in crosses numerous times, they normally fail to exhibit linkage with any of the other markers. The gene *mg* is linked to *cd* at about 20 units but to date, this linkage group has not yet been located. In all, there are six exceptions—that is, six R-distributions—which *do not involve* genes closely linked to centromeres of different chromosomes. This number (six) is in good agreement with the 7·435 expected to result from sampling error. Thus whatever mechanism is proposed to account for the excess of R-distributions, it suffices to suppose that this mechanism exerts its effect in *the neighbourhood* of the centromeres of these chromosomes.

3. NON-ADDITIVITY AND CONTRADICTIONS IN GENE ORDER

Chromosome V of *Saccharomyces* has the following structure: ur_3–centromere–*ch–*hi_1*–*is_1*–an*. The linear order of these genes was previously established by Desborough & Lindegren (1959) and Shult & Desborough (1960). The genes cu_2, *py*, and *th* frequently exhibit linkage with ur_3 and *ch*. Tetrad distributions for these genes obtained from miscellaneous crosses are presented in Table 4.

Any attempt to incorporate the genes cu_2, *py* and *th* into the chromosome V gene sequence, soon leads to contradictions in gene order. Some of these cases have been described previously. For example, in the study of Shult & Desborough (1960), Family 108, consisting of 194 tetrads, gave the gene order ur_3*–ch–*hi_1*–*is_1*–an*, as determined by 30 independent tests of linear order. By similar criteria, the consistent gene order *th–ch–*hi_1*–*is_1*–an* was obtained. Thus we should expect one of the two gene arrangements, ur_3*–th–ch* or *th–*ur_3*–ch*, to hold. However, the data for ur_3,

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th and *ch* were significantly non-additive in all three possible gene-orders.* In fact, the two 'permissible' orders listed above gave the largest value of chi-square. Again, in the total data for ur_3, *ch* and *th*, Desborough & Lindegren (1959) observed significant non-additivity in all three possible arrangements of these genes.

Table 4. *Tetrad distributions involving the genes* ur_3, ch, th, py *and* cu_2. *The total number of tetrads varies because the number of crosses carrying different gene combinations varies. For example, only one family was heterozygous simultaneously for both* py *and* cu_2.

	PD	NPD	T
ur_3–*ch*	397	18	451
ur_3–cu_2	63	16	138
ur_3–*th*	119	21	172
ur_3–*py*	55	17	95
ch–cu_2	82	8	94
ch–*th*	276	8	174
ch–*py*	176	2	61
cu_2–*th*	61	5	75
cu_2–*py*	26	4	45
py–*th*	125	6	88

4. THE AFFINITY HYPOTHESIS

Michie (1953) and Wallace (1953) proposed that preferentially segregating chromosomes carry 'sites of affinity' which are of two types, α and β. Sites of the same type are attracted preferentially to the same first division pole. Consequently genes located near these sites will tend to exhibit recombination frequencies different from 50%. In a 'convergent' zygote ($\alpha\alpha/\beta\beta$) these genes will exhibit quasi-linkage while in a 'divergent' zygote ($\alpha\beta/\beta\alpha$) these genes will exhibit greater than 50% recombination, a situation which has been variously termed as 'reverse linkage' or a 'reversal'.

The genetic analysis of the phenomenon would be greatly simplified if it were known that the sites which exhibit 'affinity' always segregate at the first division. So far, it has not been possible in the house mouse to prove conclusively that these sites are the centromeres. However, it has been possible to interpret data, without inconsistencies, on the assumption that the sites of 'affinity' are the centromeres (Wallace, 1958).

If the sites of affinity are not at the centromeres, the situation is much more complicated, for it would then be possible to obtain $\alpha\beta$ bivalents. Presumably, these would be neutral with respect to other bivalents during their sojourn through the first division, but the second division would provide a new opportunity for preferential segregation. In the case of maize, it is evident from Anaphase I bivalents that the 'neocentromeres' cannot be identified with the normal centromeres and that they exhibit second division segregation. Further their activity can be observed at both the first and second division.

* For the statistical test of linearity see Shult and Desborough (1960), pp. 176–177.

Again, if the sites are not at the centromeres, and the preferential segregation between non-homologous chromosomes is due to 'mutual' affinity, rather than affinity to a common pole (as in the case of maize and *Bombardia lunata*, Catcheside (1944)), it would then be possible for a chromosome to carry a number of affinity sites, one for each chromosome to which it is attracted.*

Michie and Wallace's assumption that the sites can be identified with the centromeres is not actually demanded by the theory, but, as can be seen from the above considerations, it certainly leads to a much simpler theory. Further, it may well be that neocentric activity observed in maize and rye (Prakken & Muntzing, 1942; Ostergren & Prakken, 1946) represents a phenomenon quite distinct from affinity.

Basically, the core of the theory depends upon the notion of a permanent 'centrotype' (α or β). This implies that preferential segregation is inheritable—or, as Wallace stated it, affinity is 'intrinsic' rather than 'extrinsic'. Conceivably, both possibilities exist: (*a*) preferential segregation is a property retained by the *bodies being segregated* and (*b*) preferential segregation is due to some mechanism which lies *outside* the chromosomes and may or may not be inherited. (A definitive proof that preferential segregation patterns in *Saccharomyces* follow hypothesis (*a*) is contained in the following section.)

5. PREFERENTIAL SEGREGATION IN FAMILY 108

As was noted in section 3, the tetrad distributions for the genes ur_3, *ch* and *th* departed significantly from what would be expected on the assumption of any one of the three possible linear orders. Preferential segregation adequately accounts for

Table 5. *Reverse linkage in hybrid* 11189 × 20704

	Tetrad distribution		
Gene combination	PD	NPD	T
ch–th	1	9	4
th–py	9	1	3
ch–py	0	10	4

No. tetrads dissected = 18, spore germination = 90%

this situation since two of the apparent linkage relationships are actually quasi-linkages, so that the chromosomal continum upon which recombination occurs consist of the three centromere-proximal (or at least site-proximal) regions. Thus we must conclude that of the two genes, ur_3 and *th*, at least one is not located on the *ch* chromosome (chromosome V). Data from a hybrid, 11189 × 20704 (Table 5), indicate that it is *th* that is not on chromosome V. Although preferential segregation of *th* and *ch* is still manifest, it favours the *non-parental* combination of chromosomes. On an affinity hypothesis, the hybrid 11189 × 20704 would be a 'divergent' zygote. In contrast to this, ur_3 has consistently exhibited linkage with *ch*, without exception (see Table 19, Desborough & Lindegren, 1959).

* For a full discussion of 'mutual' versus 'polar' affinity see Michie (1955).

Data from six families obtained since the 1959 survey (totalling more than 400 tetrads) also confirm this linkage. This is also in accord with Hawthorne and Mortimer, who place ur_3 on chromosome V. Consequently, *th* is located on a chromosome distinct from chromosome V, and in the bulk of the data these two chromosomes segregate preferentially. The quasi-linkage of *th* with members of chromosome V is apparent in the tetrad distributions from Family 108 given in Table 6.

Table 6. *Tetrad distributions of* th *and genes on chromosome V*

Gene combination	PD	NPD	T	a_3
th–ur$_3$	64	3	69	0·507
th–ch	82	0	62	0·430
th–hi$_1$	49	6	73	0·570
th–is$_1$	39	9	80	0·625
th–an$_1$	28	13	88	0·682

Note that *th* shows its strongest quasi-linkage with *ch*. As one proceeds along the right arm of chromosome V, through *ch*, *hi*$_1$, *is*$_1$, and *an*, this quasi-linkage becomes progressively weaker and weaker. The ascending NPD and tetratype frequencies (a_3) show that *th* does not preferentially segregate with chromosome V as a whole, but rather it segregates with some small region, or point, in the vicinity of *ch*, and that the distal genes *hi*$_1$, *is*$_1$ and *an* recombine with this point at progressively increasing frequencies. This is analogous to a case of preferential segregation in maize in that it implicates a specific preferentially segregating site. Thus in discussing the *C–Sh–Wx* portion of chromosome 9 in *Zea mays*, Rhoades (1952) states: 'When plants of knob-C/knobless-c constitution, which were also heterozygous for abnormal 10, were pollinated by recessive c, 64 per cent of the functioning megaspores possessed the C allele. The Sh locus, close to C, showed a similar degree of preferential segregation in comparable tests, but the Wx locus was little affected. Such a progressive decrease in effect is expected if the terminal knob on the short arm is instrumental in producing preferential segregation.'

The frequency with which preferentially segregating sites segregate in parental combination to the same pole can be determined from the formula:

$$p = \frac{1}{2}\left(1 + \frac{a_1 - a_2}{e^{-\lambda}}\right) \qquad (1)$$

where $\lambda = 2/3 \ln (1 - 3a_3/2)$ and a_1, a_2, and a_3 denote the relative frequency of PD, NPD and tetratype tetrads, respectively (see Appendix II, Shult & Desborough, 1960). This formula presumes that the sites segregate at the first division and that interference is absent. Any deviation from these assumptions will make the estimation of p in error. Application of this formula to the *th–ch* tetrad distribution yields a value of p near 100%. If the preferentially segregating sites were some distance from the centromere and/or if p were markedly less than 100%, some NPD tetrads would be expected for *th* and *ch*. Since none was observed, we may conclude that preferential segregation of sites is nearly complete and that the sites themselves are

at least in the neighbourhood of the centromeres. Evidence for the latter view was obtained by determining the position of the site on the basis of ditype frequencies and comparing this with the position of the centromere as determined from the tetratype frequencies by the method of Lindegren (1949) and Whitehouse (1956). On this basis, the site of preferential segregation was found to be located 3·7 units from the centromere (Shult & Desborough, 1960). Although various effects may introduce some error in such an analysis it is at least safe to state that the site on chromosome V to which the *th* arm is directed is at least in the neighbourhood of a centromere.

It will be noted from Table 2 that cu_2 exhibits a striking instance of reverse linkage with *ch* in Family 108. The tetrad data for cu_2 and genes of chromosome V are given in Table 7.

Table 7. *Tetrad distributions of* cu_2 *with genes on chromosome V*

Gene combination	PD	NPD	T	a_3
cu_2–ur_3	12	35	88	0·651
cu_2–*ch*	6	48	56	0·509
cu_2–hi_1	11	49	73	0·548
cu_2–is_1	12	35	84	0·641
cu_2–*an*	15	28	87	0·669

Reverse linkage is strongest with *ch* and becomes progressively weaker with genes hi_1, is_1 and *an*, distal to *ch*. This indicates that the arm bearing cu_2 preferentially segregates in non-parental combination with a fixed site (or small region) close to *ch*, and that other genes of chromosome V recombine with this site at progressively increasing frequencies according to their distance from *ch*.

Application of formula (1) to the tetrad distributions for cu_2 and *ch* indicates that the sites segregate in parental combination in only 0·3% of the meioses. (The error associated with this estimate is somewhat larger than that for *th*–*ch* since, here, the tetratype frequency is larger and there is some indication of intraregional interference.)

Assuming nearly 100% 'repulsion' of the sites, the tetrad distributions for ur_3, *ch* and cu_2, indicate the gene 'order' ur_3–*ch*–cu_2—i.e. recombinations between ur_3 and cu_2 are the results of independently occurring recombinations in ur_3–*ch* and *ch*–cu_2. (This is actually the *genetic* definition of 'order', Shult & Lindegren, 1955.) In terms of chromosome structure this means that the preferentially segregating site with which the cu_2 arm segregates is either at the *ch* locus or distal to it. This finding again raises two questions mentioned earlier: Are the sites centromeres and if not, does a chromosome carry multiplicity of sites, each having affinity for different chromosomes?

On an affinity hypothesis, Family 108 consists of tetrads from a 'divergent' zygote (α–*ch*, β–CU_2/β–*CH*, α–cu_2). If, as the data suggest, α segregates with α in nearly all meioses, then most of the 48 NPD tetrads (*CH* CU_2, *CH* CU_2, *ch* cu_2, *ch* cu_2) contain spores with either α–*ch*, α–cu_2 or β–*CH*, β–CU_2 combinations of chromosomes. A

non-parental ditype tetrad for *ch* and cu_2 was selected for further mating and is given in Fig. 1. This tetrad is a little unusual in that there is also no crossing-over between *ch* and ur_3 (so that the three centromere-proximal regions bordering *ch*, ur_3 and cu_2 are likely to contain only parental material) and mating-type segregates in tetratype pattern relative to ur_3, *ch* and cu_2. Two of the segregants differing in mating-type were chosen as parents of a new family, Family 118. In the new family *CH* and CU_2 exhibit nearly 100% preferential segregation in the *parental* combination (see Table 8). This is as would be expected if the *centre of attraction segregates with the body being attracted*—in particular, if these are one and the same. It would not be expected if the centre of control over preferential segregation was a mechanism *outside* (or extrinsic to) the bodies being segregated, for if the mechanism were inherited, another R-distribution should result in Family 118, while if it were not inherited, segregation at meiosis I would be random.

Table 8

(*a*) *Tetrad distribution of Family* 108

ur_3–*ch*	85– 1–79
ur_3–cu_2	12–35–88
ch–cu_2	6–48–56

Parents of 108: 21708 *a* ur_3 *CH TH* cu_2 × 22541 *α* UR_3 *ch th* CU_2

23342 *α ur CH CU*
23343 *a UR ch cu* } parents of 118
23344 *a UR ch cu*
23345 *α ur CH CU*

(*b*) *Tetrad distribution of Family* 118

ur_3–*ch*	12–0–17
ur_3–cu_2	12–4–14
ch–cu_2	13–1–13

6. REVERSE LINKAGE IN FAMILY 217

Copper mutants (M-901-34C, H-1323-23A, H-1385-4A) were kindly supplied us by Dr Mortimer and Dr Hawthorne. These mutants do not complement and have been given the gene designation, cu_1, by these authors. In crosses with *ch* this mutant failed to exhibit the preferential segregation which had been obtained consistently heretofore with the mutant cu_2 described above. Hawthorne and Mortimer observed linkage between cu_1 and the centromere-linked gene ar_4, while the gene cu_2 failed to exhibit linkage with ar_4 (Family 206, cu_2–ar_4, 0–1–7). Subsequently, cu_2 and cu_1 were found to complement in all cases while selfings did not, indicating that these are distinct loci. The hybrid (31592 × 32418) which gave rise to Family 217 was heterozygous for ar_4 and cu_1. The tetrad distribution for ar_4 and cu_1 consisted of only 1 parental ditype tetrad, 18 non-parental ditype tetrads and 21 tetratype tetrads (6 tetrads could not be classified). The parents of this hybrid were obtained from Mendelian segregating tetrads, and their phenotypes were retested three times.

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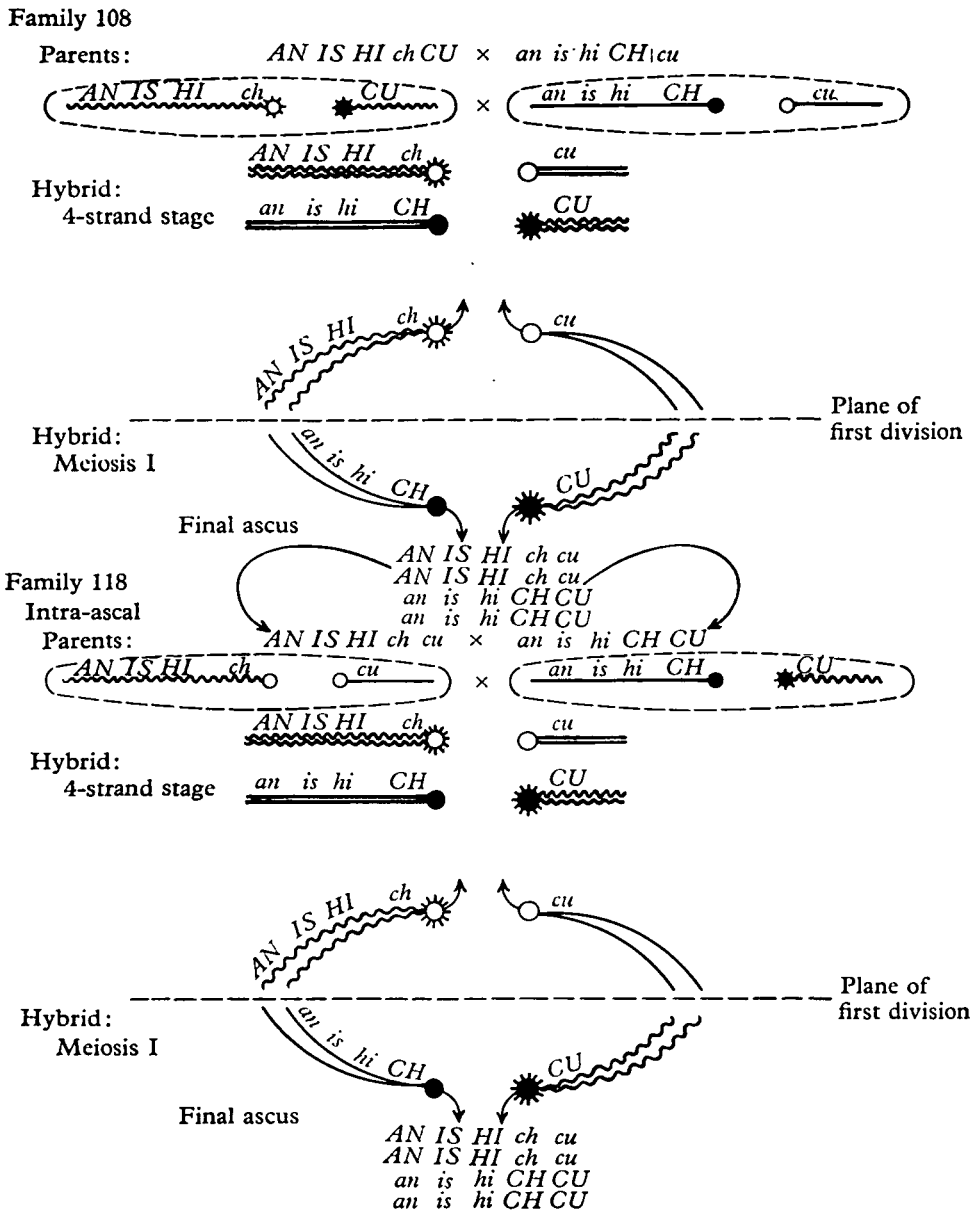

Fig. 1. Preferential segregation of chromosome V (*ch–hi*$_1$–*is*$_1$–*an*) with a chromosome carrying *cu*$_2$ in two families. Rough and smooth chromosomes denote their genetic origin in these families. Centromeres which preferentially segregate are either both light or both dark bodies. The parents of Family 108 carry centromeres of opposite colour—that is, they are reciprocally heterocentric—so that preferential segregation at meiosis I, leads to a non-parental combination of centromeres in the progeny. Two members of an ascus carrying this non-parental combination were mated to yield Family 118. The types of centromeres and the origin of the chromosomes in these cultures is depicted in the centre of the figure. Here preferential segregation of light centromeres with light centromeres, produces a parental combination of centromeres in the progeny. Consequently, in Family 108, most of the tetrads are non-parental ditype for *cu*$_2$ and *ch*, while in Family 118, the parental ditype tetrads predominate.

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Such a distribution is inexplicable on the assumption that cu_1 and ar_4 are located on the same chromosome and the data are completely at odds with those of Hawthorne & Mortimer (1960). However, the affinity hypothesis serves to reconcile the two findings. The reverse linkage recorded in Family 217 results from the fact that 31592 × 32418 is a 'divergent' zygote, while (if affinity is intrinsic) the quasi-linkage evident in the data of Hawthorne and Mortimer results from a 'convergent' zygote.

This view is supported by the finding that for the genes cu_1 and ar_4 affinity was intrinsic, as was the case with cu_2 and *ch*. In particular, it was even possible to duplicate the data of Mortimer and Hawthorne by the use of intra-ascal matings. Two intra-ascal hybrids were constructed from one of the eighteen tetrads which were NPD for cu_1 and ar_4 (see Table 9). Although the number of tetrads involved was quite small, the results of the two matings were consistent and the total tetrad distribution for cu_1 and ar_4 exhibits quasi-linkage.

Table 9. *Tetrad distributions and mating schema for progeny of Family* 217

31592	α	+	ar_4	+	} parents of Family 217
32418	a	ur_3	+	cu_1	}
			cu_1–ar$_4$	1:18:21	

one of 18 NPD tetrads:

32781	α	+	ar_4	cu_1	} parents of Family 219
32782	a	ur_3	+	+	}
32783	a	ur_3	+	+	} parents of Family 218
32784	α	+	ar_4	cu_1	}
	cu_1–ar_4		4:1: 8	Family 218	
			4:0:10	Family 219	
			8:1:18	Total	

The single PD tetrad:

32808	a	ur_3	ar_4	+ ↘	
32809	α	+	+	cu_1	parents of Family 220
32810	α	ur_3	+	cu_1 ↗	
(did not germinate)					
	cu_1–ar_4		5:2:14	Family 220	

The appearance of only one PD tetrad in the case of the cu_1–ar_4 reverse linkage in Family 217, suggests that preferential segregation of sites is nearly complete, say 0 to 15% parental combinations. This raises the following question: How did the single PD tetrad arise? There are three possibilities: (1) The preferentially segregating sites failed to go to the same pole in that meiosis. On this theory, each spore of this tetrad still retains an $\alpha\beta$ or $\beta\alpha$ combination of sites. (2) The PD tetrad may have arisen solely on the basis of double exchange. In this case, each spore would carry the usual $\alpha\alpha$ or $\beta\beta$ combination of sites. (3) It is possible that the capacity for preferential segregation was lost prior to the first division, so that the PD tetrad

results from random assortment. These three hypotheses can be tested by means of an intra-ascal mating heterozygous for both cu_1 and ar_4 in the PD tetrad. On hypothesis (1), reverse linkage should occur in this cross because of the $\alpha\beta$ and $\beta\alpha$ combination of sites in each parent. On hypothesis (2), quasi-linkage should result because of the homocentric combination of sites. On hypothesis (3), equal numbers of PD and NPD tetrads should be obtained because of the loss of any preferential control over assortment.

This intra-ascal hydrid (32808 × 32810) was constructed and the resultant ratio of tetrad types for cu_1 and ar_4 was 5:2:14 (3 additional tetrads exhibited irregular segregation and in one other no spores germinated). Although this distribution does not depart significantly from either quasi-linkage or random assortment, it does deviate (by Binomial statistics) from reverse linkage of the magnitude expected: 1:18:21 for Family 217 ($p = 7{\cdot}6 \times 10^{-6}$) or 2:9:20 predicted from Families 218 and 219 ($p = 1{\cdot}54 \times 10^{-5}$). This rules out hypothesis (1), the failure of active sites to segregate preferentially, but the number of tetrads is too small to obtain a decision between hypotheses (2) and (3).

SUMMARY

In tetrad data obtained from the Carbondale yeast stock, instances of greater than 50% recombination occur far more frequently than would be expected by chance. In the main, genes exhibiting this effect are located in the vicinity of the centromeres. Difficulties in assigning linear order also persist throughout the total data for certain gene combinations. The affinity hypothesis suffices to account for these two effects; the former resulting from 'divergent' combinations of affinity sites in the zygote, the latter, representing nonlinear quasi-linkages resulting from 'convergent' combinations.

Chromosome V (ur_3–centromere–*ch*–hi_1–is_1–*an*) exhibits quasi-linkage with the gene *th* and reverse-linkage with the gene cu_2, in Family 108. In each case 'linkage' is strongest with *ch* and becomes progressively weaker with the genes hi_1, is_1 and *an*, respectively, indicating that the preferentially segregating sites involved lie close to *ch*. It was impossible to determine whether the two sites were identical, as would be expected on an hypothesis of 'polar' rather than 'mutual' affinity.

Intra-ascal matings within tetrads NPD for cu_2–*ch*, yielded quasi-linkage for these genes in F_2, showing that the attraction is retained by the sites being segregated.

A second case of reverse linkage for cu_1 and ar_4, in Family 217, gave a similar effect —i.e. reverse linkage in F_1, becoming quasi-linkage in the NPD—intra-ascal F_2.

An intra-ascal mating between members of the single PD tetrad for cu_1 and ar_4 in Family 217 indicated that this tetrad did not result from 'chance' failure of otherwise active sites to preferentially segregate.

The authors wish to acknowledge the technical assistance of Mr Yuh Lin Hwang for his help in preparing the Tables. They are also deeply indebted to Mrs Lindegren and her staff for all the data from which the conclusions were drawn.

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