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AFFINITY: EVIDENCE FROM CROSSING INBRED LINES OF MICE

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1. INTRODUCTION

IN order to test the theory of affinity (Michie, 1953; Wallace, 1953), an outcrossing programme in mice was set up in 1951 and completed in 1954. Definitions of the terms used in affinity work have been given (Wallace, 1958*a*, chap. II); short notes only need be given here. An outline of its design has also been given (*ibid.*): this will be considered in more detail here.

(i) *Definitions*

Quasi-linkage: the non-random segregation of markers which ordinarily segregate independently.

The theory of affinity: there can be more than one kind of centromere: when an organism contains two kinds in each of at least two pairs of homologous chromosomes, then, at the first division of meiosis, similar centromeres tend to travel to the same pole. This causes markers near them to segregate as if linked. Thus affinity explains quasi-linkage.

Centrotype: centromeric constitution.

Heterocentricity: the presence of two kinds of centromere within a homologous pair of chromosomes. Symbolised α/β. Analogous to heterozygosity.

Homocentricity: the presence of only one kind of centromere within a homologous pair of chromosomes. Symbolised α/α or β/β. Analogous to homozygosity.

Homogeneously homocentric: the presence of one kind of centromere within a homologous pair, and of a similar kind within other pairs. Symbolised α/α, α/α, etc.

Heterogeneously homocentric: the presence of one kind of centromere within each homologous pair, differing between pairs. Symbolised α/α, β/β, etc.

Convergent heterocentric: a heterocentric in which like centromeres come from the same parent. Symbolised $\frac{\alpha\ \alpha}{\beta\ \beta}$. Analogous to coupling. Markers segregate according to the phase of their " linkage ". A convergent heterocentric will result if the parental stocks differ but are each homogeneously homocentric.

Divergent heterocentric: like centromeres come from different parents. Symbolized $\frac{\alpha\ \beta}{\beta\ \alpha}$. Analogous to repulsion. Markers segregate as if they were in the opposite phase (those in coupling, as if in repulsion; repulsion, as if in coupling). Such a segregation is a " reversal " and gives a recombination value formally exceeding 50 per cent. A divergent heterocentric will result if the parental stocks differ, and are each heterogeneously homocentric.

Types of centromeres: It is in theory to be expected that centromeres of the same natural inbreeding population will generally be similar. Laboratory mice probably stem from several such populations and may well have many types of centromere. It is conceivable that those of certain chromosomes may exhibit preferential segregation more strongly than others (*cf.* the " knob " of chromosome

10 in maize, Longley, 1945). For simplicity and because there is no evidence as yet to the contrary, only two kinds and equal preferential tendencies are assumed.

Separation value: the degree to which similar centromeres fail to pass to the same pole. Analogous to recombination value. Similar centromeres are said to " attract ".

" Polar " and " mutual " attraction: attraction can be thought of as occurring between the centromere and the pole, or between one centromere and another (Michie, 1955*a*). It is not necessary here to distinguish between these possibilities.

(ii) *Design*

The aim of the programme was to produce data on the segregation of markers of different linkage groups from supposedly heterocentric heterozygotes, and to compare these with data for the same markers from supposedly homocentric heterozygotes. The former are expected to show quasi-linkage and the latter independence, the contrast providing clear evidence of affinity.

Homocentricity, like homozygosity, may be expected as a result of inbreeding. Data supposedly from homocentrics is therefore drawn entirely from inbred material.

Heterocentricity, like heterozygosity, may be expected in the F_1 of outcrosses. Accordingly, multiply-heterozygous F_1 were made up from crosses between multiply-mutant stocks. However, in contrast to homocentricity and homozygosity, while this is undoubtedly a good place to look for it, it does not invariably result. It results only when the stocks used in the outcross differ in their centrotype, *i.e.* in doubly heterocentric heterozygotes. Since centromeres have no effect on the phenotype, there is no way of knowing, before making the crosses, which stocks differ; difference can only be deduced from the appearance of quasi-linkage in the F_1, *i.e.* after the crosses are made. Heterozygotes from stocks which are similar in centrotype for one or both of two pairs of homologues, will be partially or completely homocentric and so will show independent assortment of the markers.

Some quasi-linkages are expected to be reversals (from divergent heterocentrics) and others to give values less than 50 per cent. (from convergent heterocentrics). This feature may be expected to increase heterogeneity. Loose linkage is a hypothesis which, for many markers in different linkage groups, remains a possibility which must be removed if quasi-linkage is to be accepted. Reversal and heterogeneity here do just this, as does independence in the inbred material. Moreover, these features conjointly militate strongly against an interpretation based on viability or other interactions, and against translocation.

The programme demonstrates affinity, then, if

(1) outbred heterozygotes give heterogeneous data, and
(2) individual heterozygotes can be identified from such heterogeneous data as showing quasi-linkage, or
(3) the data from outbred heterozygotes give an overall departure from independence (with or without heterogeneity), and
(4) inbred heterozygotes homogeneously give independence.

In order to achieve significance, it is necessary that statistically the greatest " amount of information " is given by the data, and that as far as possible, only markers likely to be near their centromeres are used. Accordingly, all matings were backcrosses of the multiple heterozygotes to multiple recessives, and in the main, only markers which had previously been involved in quasi-linkages were used.

(iii) *Further uses of this design*

This design, namely outcrossing with multiply-mutant stocks and backcrossing the heterozygous F_1 to the multiple recessive, has certain features worth further mention.

If the stocks used in the outcrosses are themselves inbred, all heterozygotes from crosses of the same stocks, should give the same kind of segregation—whether independence or either kind of quasi-linkage. Conversely, if there is heterogeneity between the performances of such heterozygotes, there is reason to suppose that some centromeres are still segregating despite the inbreeding. In an inbred line with enforced segregation of markers, segregation not only of their centromeres may be expected, but also of the centromeres of some unmarked chromosomes —if there is a " cumulative attraction " (Wallace, 1958*a*, p. 219). Heterogeneity tests are thus a test of this possibility. Unfortunately the lines used in this programme were not suitable, for, though inbred for more than ten years, new factors had recently been introduced to most of them (by an " upgrading " technique): when the crosses were made, only one line had a history of more than eight generations of sib-mating. (It is regretted that the claim to have demonstrated " cumulative attraction " (*ibid.*, p. 216) was premature: the small amount of inbreeding was not then realised).

Another possibility, which the use of several inbred stocks may be able to discern, is whether or no there are more than two types of centromere. If homocentricity within each of more than two stocks can be assured, it is conceivable that two markers will give quasi-linkages which, on the basis of only two kinds, do not allow of a consistent specification of the centrotype of all the stocks used.

These, and other possibilities, such as discrimination between " polar " and " mutual " affinity, and tests for differing separation values, need further theoretical and experimental study.

(iv) *Material*

The twenty-one segregating inbred lines of Fisher (1949, chap. II) are ideal, both for the provision of multiple-mutants for outcrossing, and as a source of the control inbred data.

When the programme was started (1951), the evidence was in favour of the following as centromere-markers (in descending order

of strength of evidence): Danforth's short-tail (*Sd*, V), Caracul (*Ca*, VI), short-ear and Maltese dilution (*se*, *d*, II) and brown (*b*, VIII). (*se* and *d* are considered as one marker because of very close linkage). Accordingly, the Segregating Inbred Lines and two other relatively inbred stocks were used, with preference for those containing these four markers. All the factors available in each are listed below, since there was a possibility that new centromere-markers would reveal themselves.

Line 2:	*Sd A* and *a*, *Ca*, *s*, *ln*, *se*.
Line 4:	*Sd a*, *py fz*, *b*, *se d*.
Line 7:	*Re*, *py*, c^{ch}, *s*, *a*.
Line 10:	*Ca bt*, *m b*, *a*.
Elite poly (E):	*py ln*, *pa a*, *b*.
Agoutis (G):	A^{y} A^{w} *A* a^{t} *a* (*A* locus alleles).

The agouti locus (*A*, *a* etc.) was the only one not segregating, being homozygous in all stocks except Line 2 and Agoutis (G).

s = recessive pied, III; *fz* = fuzzy, *ln* = leaden, *py* = polydactyly, XIII; *Re* = rex, VII; c^{ch} = chinchilla, I; *bt* = belted, VI; *m* = misty, VIII; *pa* = pallid, V; the absence of a comma indicates linkage.

The multiple recessives, to which the F_1 of the outcrosses were mated, had sometimes to be specially constructed and often suffered reduced fertility; in many cases animals not fully recessive were therefore used. For this reason the totals from the backcrosses are often different between two-points within the same heterozygote (tables 4, 5, and 6).

Not more than 30-50 young classified for several factors may in general be expected from a single outbred female. This is not sufficient to show significantly the small deviations from independence which quasi-linkage is usually expected to comprise. Male heterozygotes only were therefore used in the backcrosses, in polygamous matings, and data for two-points from any one heterozygote, consisting of less than 50 young classified for those two points, are excluded (tables 4, 5, 6, 7 and 8). This removes a large quantity of data, in most experiments a wasteful procedure, but the results appear to justify it. No rejections were made, however, from the inbred and outcross matings (tables 2 and 3), because they were necessarily mainly monogamous and seldom bred as many as 50 young.

Data for *py* are excluded because, as was expected after outcrossing, penetrance was too poor for a reliable interpretation to be made. Segregations with sex are omitted because they have not been summarised as yet; there were no obvious anomalies.

2. IDENTIFICATION OF CENTROMERE-MARKERS

It was made a condition of acceptance of data as identifying centromere-markers, that the data be of a similar nature as those sought

in the present experiment; namely that quasi-linkages involving the markers, or heterogeneity in "linkage" data, occur in outbred material, and that inbred material (where available) show independence. It is striking that a search for quasi-linkage in the literature and unpublished records in the Department, revealed many instances (Wallace, 1954, 1958*b*), and that *all* of them fulfilled these conditions.

A review of the literature (to 1959) concerning the chosen markers *Sd*, *Ca*, *se d*, and *b*, and an acccount of unpublished records serves as a summary of the evidence in their favour. As such, it is clearly of value also as part of the general evidence for affinity.

(i) *Evidence from the literature*

The mutants *d* and *b* (with *s* and waltzer, *v*), occur in the data which first prompted the theory of centromeric attraction (Michie, 1953). These were from the F_1 of a subspecific cross. *d* gives the strongest associations.

The mutants *Sd*, *fi* (fidget, also V), and *Ca* (with dominant pied, *W*, III) occur in the first data from laboratory stocks (Wallace, 1953, 1958*a*). As the segregation of *Sd* was not published, the full segregation and analysis is now given (table 1A and section 4 (i)); agreement with an affinity interpretation is found to extend beyond its occurrence in outbred material. *Sd* and *fi* (with a^t and *W*) were then used in a critical test of affinity (Wallace, 1957*b*, 1958*a*, 1959), from which the centromere is located between the two.

That the sporadic quasi-linkages and heterogeneous "linkage" data found in the literature are from outbred material is either explicit in the report or implicit in that the factors are being tested for linkage for the first time. The sporadic cases are: *Sd* and *Ca* (Dunn *et al.*, 1940); *Ca* (with *vt* and *a*) (Michie, 1955*b*); *b* (with W^v) (*ibid.*); *b* and *d* (with Alopecia, *Al*) (Dickie, 1955); and *d* (with wavy-1, *wa*—1, XI) (Fisher and Mather, 1936*a*, *b*). The heterogeneous "linkage" data concern: *b* (with *wa*—1) (*ibid.*); and *d* (with albinism, *c*, and pink-eyed dilution, *p*, I, *wa*—1, and dominant White, Mi^{wh}, XI) (Carter and Falconer, 1952, 1953).

Recent data for *Sd* from material bred by Fisher and reported by Parsons (1959) are grouped according to the initial outbred generation and subsequent stages of inbreeding. Here *Sd* with Splotch, *Sp*, and *ln*, XIII) exhibits significant single quasi-linkages and heterogeneity. Centromere position in XIII is discussed.

Finally, *Ca* and *b* show quasi-linkage in a new interspecific cross with several factors (Chatterley, 1958). The association remains significant when the chance of obtaining it as one among the several possible ones is considered. This assessment of significance is, naturally, seldom available for the sporadic cases.

TABLE 1

Quasi-linkage backcross data for V markers with three independent markers

A: *W* (III), *Ca* (VI), *fi* and *Sd* (V)

Genotype and origin of heterozygotes	Three-point segregations: modes of gamete formation										Recombination values (per cent.)	Independence tests
Outbred . . .	(P)	(*fi*)	(*Ca*)	(*W*)	Total	(P)	(*Sd*)	(*Ca*)	(*W*)	Total		
2♀♀ : *fi*/*SdCaW* . .	40	23	25	29	112	39	24	27	22	112	*fi*-*Sd* = 24·11 *Ca*-*W* = 43·75 *Ca*-*fi* = 42·86 *Ca*-*Sd* = 45·54 *W*-*fi* = 41·96 *W*-*Sd* = 41·07	Is (40) : (23+25+29) = 1 : 3? $\chi^2_1 = 6{\cdot}8511$ Is (39) : (24+27+22) = 1 : 3? $\chi^2_1 = 5{\cdot}7619$
	The slight discrepancy between these figures and those published (Wallace, 1958*a*) is due to an error in the compilation of the earlier work.											

B : *Sp* (XIII), a^t, *fi* and *Sd* (V)

Genotype and origin of heterozygotes	Two-point segregations: genotypes run vertically															Recombination values (per cent.)	Independence tests Do recombs. = non-recombs. ?
	Sp / a^t	*Sp* / *a*	+ / a^t	+ / *a*	Total	*Sp* / +	*Sp* / *fi*	+ / +	+ / *fi*	Total	*Sp* / *Sd*	*Sp* / +	+ / *Sd*	+ / +	Total		
a. Outbred (Bar Harbor) 1 ♂ : *Sp*/*Sd* . .	—	—	—	—	—	—	—	—	—	—	29	64	39	38	170	*Sp*-*Sd* = 39·41	$\chi^2_1 = 7{\cdot}6235$
b. Outbred (Cambridge) 1 ♂+2 ♀♀ : *Spa*/a^t*fiSd*	63	80	80	81	304	96	47	84	77	304	44	99	79	82	304	*Sp*-a^t = 47·37 *Sp*-*fi* = 43·09 *Sp*-*Sd* = 41·45	$\chi^2_1 = 0{\cdot}8421$ $\chi^2_1 = 5{\cdot}8028$ $\chi^2_1 = 8{\cdot}8947$
c. Inbred (Cambridge) 1 ♂ : *SpSd*/a^t*fi* . .	14	19	23	31	87	19	14	33	21	87	13	20	28	26	87	*Sp*-a^t = 51·72 *Sp*-*fi* = 54·02 *Sp*-*Sd* = 55·17	$\chi^2_1 = 0{\cdot}1034$ $\chi^2_1 = 0{\cdot}5632$ $\chi^2_1 = 0{\cdot}9310$

(ii) *Evidence from unpublished records*

The combination of factors from different stocks to start a segregating inbred line clearly involves outbreeding. Accordingly quasi-linkage should be confined to the early generations, all the later, inbred, generations showing independence. A summary of the segregations concerning *Sd* with *p* and *Ca* reveals exactly this (tables 2A and 2B): the outbred heterozygotes have the high χ^2 values, the inbred's values are low.

Similarly linkage-testing crosses are usually from outbred material, subsequent tests from relatively inbred material. Here again, *Sd* and *fi* appear in quasi-linkages in the outbred: *Sd* was in fact thought to be linked with *Sp*, both in Bar Harbor (data of Diane Kelton, table 1B*a*) and in Cambridge (table 1B*b*) until later work in both laboratories placed *Sp* centrally in XIII (see Parsons, 1958). The more inbred data (table 1B*c*) shows independence. As with the associations of *Sd* and *fi* with *W*, further analysis of those with *Sp* (section 4 (i)) reveals a fuller agreement with an affinity interpretation.

Finally, *b* has been involved in data from outbred material with *fi* which, though of a rather different kind from the foregoing, can most simply be explained as quasi-linkage. The viability of *fi* is always much reduced after an outcross to an unselected stock (Wallace, 1957*a*), but no case of extremely low viability of *b* has been reported elsewhere or has been found in our stocks. The paucity of *b* as well as of *fi*, observed in a coupling intercross (table 2C*a*), therefore requires a special explanation. The intercross followed an outcross; if it is assumed that *fi* and *b* are showing quasi-linkage, the apparent inviability of *b* is then easily explained as due to its association with *fi* which is suffering real inviability. This explanation is borne out by data from the coupling intercrosses derived from two generations of inbreeding (table 2C*b*): *fi* is still fairly inviable, but *b* shows no quasi-linkage with *fi* or apparent inviability.

3. RESULTS

Tables 4, 5 and 6 present the backcross data from the F_1 of the outcrosses between inbred stocks (section 1 (iv)). The control data from inbred material are presented in table 2 (B*c*, B*d*, D and E), and an additional type of control data, provided by the outcrosses, in table 3. In tables 4, 5 and 6, the segregations for each two-point are arranged under the headings: one complementary pair *versus* the other complementary pair; χ^2 tests of the deviation of the observations under these headings from the 1 : 1 expected for independence, are also given. Since the centrotypes of the parental stocks are unknown (section 1 (ii)), deviation can be expected, on an affinity basis, in either direction. Linkage tests can be made by rearrangement

TABLE 2

Data for independent factors from Cambridge records

Type of mating	Origin and number of heterozygotes	Segregations					Independence tests		Recombination (per cent.)
		a b	a	b	+ +	Total	χ^2	p	
A : *Sd* (V) and *p* (I) (Line 11)									
a. C.B.	Outbred (5)	24	43	36	32	135	3·9185	<0·05	41·48
b. R.B.	Outbred (5)	26	23	27	16	92	0·6957	<0·5	
c. C.B.	Inbred (4)	25	20	29	24	98	0·0000	1·0	
B : *Sd* (V) and *Ca* (VI) (Line 2)									
a. C.B.	Outbred (5)	17	32	28	20	97	5·4536	<0·02	61·86
b. R.B.	Outbred (1)	16	11	17	15	59	0·1525	<0·7	
c. C.B.	Inbred (3)	13	12	10	14	49	0·5102	<0·5	
d. R.B.	Inbred (5)	43	42	45	43	173	0·0058	<0·95	
C : *fi* (V) and *b* (VIII) (Stock E)									
a. C.I.	Outbred (1 pair)	0	2	3	40	45	8·0667	<0·01	≏19
b. C.I.	Inbred (3 pairs)	3	12	20	52	87	0·0897	<0·8	
D : *Sd* (V) and *se* (II) (Line 2)									
a. C.B.	Inbred (9)	29	26	39	33	127	0·0709	<0·8	
b. R.B.	Inbred (5)	24	20	26	22	92	0·0000	1	
E : *Ca* (VI) and *se* (II) (Line 2)									
a. C.B.	Inbred (6)	13	17	16	19	65	0·0151	≏0·9	
b. R.B.	Inbred (6)	31	26	28	38	123	1·8293	<0·2	

Type of mating: R = repulsion, C = coupling, B = backcross, I = intercross.

Segregations: The symbol a stands for the mutant first mentioned in the relevant heading (A, B, C) and b for the second, *e.g.* under heading A, a = *Sd*, b = *p*. Any discrepancies with earlier figures are due to regrouping of data with closer attention to consanguinity.

Independence tests: Here and in subsequent tables, the single-factor ratios are homogeneous. χ^2 tests the equality of non-recombinants and recombinants except where both single-factor segregations are disturbed, when the contingency χ^2 is used. Under heading C.*a*., χ^2 tests the fit of the observed ratio $B : b$ to the expected 3 : 1 (section 2 (ii)).

of the complementary pairs as non-recombinants and recombinants; the phase of their " linkage " is given under " genotype of the heterozygote ".

(i) *Data from the F_1 of outcrosses*

The overall significance of the deviations from independence for the total body of data for *Sd*, *Ca*, *se* and *b* is given by the sum of the χ^2 values for each two-point segregation. This is:

	Sd-Ca	*Sd-se*	*Sd-b*	*Ca-se*	*Ca-b*	*b-se*	Total
χ^2	29·88	16·34	8·58	14·70	1·45	1·46	72·41
d.f.	17	9	8	8	7	2	51

χ^2 for 72·41 for 51 d.f. is significant at the 2·5 per cent. level. It is

TABLE 3

Data from the outcrosses—Sd-Ca

Genotype of outcross and number of matings	Segregation of the progeny (genotypes run vertically)					Ratio between complementary pairs	Independence tests		
	Sd Ca	*Sd*	*Ca*	+	Total	*SdCa* & ++ : *Sd* & *Ca*	χ^2	d.f.	*p*
$Sd_4 \times Ca_{10}$ (3)	14	17	22	21	74	35 : 39	0·2162		
$Sd_2 \times Ca_{10}$ (4)	16	6	11	16	49	32 : 17	4·5918		
$SdCa_2 \times ++_E$ (8)	24	27	25	28	104	52 : 52	0·0000		
$SdCa_2 \times ++_G$ (1)	10	5	10	9	34	19 : 15	0·4706		
$SdCa_2 \times ++_4$ (2)	8	13	10	19	50	40 : 36	0·2105		
$Sd_4 \times Ca_2$ (1)	7	4	9	6	26				
Total χ^2 . . .						— —	5·4891	5	
Deviation χ^2 . . .						178 : 159	1·0712	1	<0·5
Heterogeneity χ^2 . .							4·4179	4	<0·5

The suffices under " genotype " denote the Line or Stock containing the various genes.

thus clear that deviations from independence have occurred and that they cannot be accounted for on a chance basis.

The χ^2 totals for each of the three two-points involving *b* are insignificant, and analysis shows no heterogeneity even for *Sd-b*. It may therefore be concluded that *b* was probably not involved in any associations; accordingly no details of its segregations are given in the tables. On an affinity basis, this means either that, despite earlier evidence, *b* is not sufficiently close to its centromere to be able to show quasi-linkage, or that all the heterozygotes for *b* were partially or completely homocentric. The latter alternative is not unlikely, since some homocentricity was expected (section 1 (ii)); discrimination between the two alternatives cannot be made from this type of experiment.

The hypothesis of linkage, as an explanation of the deviations

from independence, will be considered for each two-point separately. That of viability disturbance as producing spurious " linkage " may be disposed of at once. Disturbance in the ratio between complementary pairs can be expected only if *both* single-factor ratios are

TABLE 4

Data from the backcrosses—Sd-Ca

Genotype and reference number of the heterozygote		Segregation of the progeny (genotypes run vertically)					Ratio between complementary pairs	Independence tests		
		Sd Ca	*Sd*	*Ca*	+	Total	*SdCa* & ++ : *Sd* & *Ca*	χ^2	d.f.	*p*
Sd_4/Ca_{10}	1a	26	21	31	55	133	81 : 52	6·3233	1	<0·02
	2a	46	39	39	43	167	89 : 78	0·7246		
	2b	24	28	36	28	116	52 : 64	1·2414		
	2c	69	51	45	55	220	124 : 96	3·5636		
	2d	89	86	95	96	336	185 : 181	0·0437		
Sd_2/Ca_{10}	3a	21	22	26	24	93	45 : 48	0·0968		
	3b	49	41	56	46	192	95 : 97	0·0208		
	4a	22	16	9	23	70	45 : 25	5·7143	1	<0·02
	4b	69	68	60	69	266	138 : 128	0·3759		
$SdCa_2$/E	5a	39	20	36	44	139	83 : 56	5·2446	1	<0·05
	6a	26	19	24	21	90	47 : 43	0·1778		
	6b	28	16	26	21	91	49 : 42	0·5385		
	7a	40	47	46	37	170	77 : 93	1·5059		
	7b	39	43	43	52	177	91 : 86	0·1412		
$SdCa_2$/G	8a	20	29	22	29	100	49 : 51	0·0400		
Sd_4/Ca_2	9a	60	53	44	65	222	125 : 97	3·5315		
	9b	32	36	37	32	137	64 : 73	0·5912		
Total χ^2								29·8751	17	
Deviation χ^2							1439 : 1310	6·0535	1	<0·02
Heterogeneity χ^2								23·8216	16	<0·1

Here and in tables 5, 6 and 9, suffices under " genotype " denote from which Line or Stock the genes came into the heterozygotes. Animals sharing a reference numeral came from the same outcross mating.

The data for animal 1a include those published in 1953.

affected. In each two-point, while one single-factor ratio is either homogeneously unequal throughout the data or occasionally heterogeneous within one or two groups (*e.g. Sd* in the Sd_4/Ca_{10} group), the other factor shows no upset of any kind. The segregation of animal 5a (table 5) is the only exception: here the contingency χ^2, testing the association between the two segregating factors, rather than the χ^2 testing equality of the complementary pairs, is used in the column

headed " Independence tests ". The conclusions drawn from the χ^2 analysis cannot therefore be biased by viability disturbance.

The hypotheses of linkage and quasi-linkage will now be considered for each two-point in turn.

The total *Sd-Ca* segregation (table 4) shows a very significant departure from independence ($\chi^2_1 = 6{\cdot}0535$). This cannot be interpreted as linkage since the ratio of non-recombinants : recombinants is 1335 : 1414. This gives a recombination value of 51·44 per cent.,

TABLE 5

Data from the backcrosses—Sd-se

Genotype and reference number of the heterozygote		Segregation of the progeny (genotypes run vertically)					Ratio between complementary pairs	Independence tests		
		Sd	*Sd se*	+	*se*	Total	*Sdse* & ++ : *Sd* & *se*	χ^2	d.f.	*p*
$Sdse_4/+_{10}$	1a	19	25	40	45	129	65 : 64	0·0078		
	2a	12	15	17	8	52	32 : 20	2·7692		
	2d	25	35	30	20	110	65 : 45	3·6364		
$Sdse_2/+_{10}$	3b	26	28	28	31	113	56 : 57	0·0088		
	4b	35	37	38	36	146	75 : 71	0·1096		
$Sdse_2/+_E$	5a	10	10	27	9	56	37 : 19	2·5562		
	7b	35	29	32	48	144	61 : 83	3·3611		
$Sdsed_4/+_2$	9a	59	35	50	53	197	85 : 112	3·7005		
$Sdsed_4/+_7$	10a	22	20	25	19	86	45 : 41	0·1860		
Total χ^2								16·3356	9	
Deviation χ^2							521 : 512	0·0784	1	<0·8
Heterogeneity χ^2								16·2572	8	<0·05

Here and in table 6 *se* stands for *se* or *d* or both : there were no *se*/*d* crossovers.

and the heterogeneity χ^2 becomes 27·6048 for 16 d.f. with a probability less than 0·05. The alternative of quasi-linkage is more acceptable. Returning to the arrangement of the complementary pairs in the table, the subsignificant heterogeneity χ^2 of 23·8216 for 16 d.f. leaves some doubt as to whether all segregations are showing it or only some. In all groups except the $SdCa_2$/E and $SdCa_2$/G, a quasi-linkage is a reversal.

The *Sd-se* segregation (table 5) shows no departure from independence ($\chi^2_1 = 0{\cdot}0784$), but there is significant heterogeneity ($\chi^2 = 16{\cdot}2572$ for 8 d.f. with a probability less than 0·05). All

matings are, for linkage purposes, of the same phase (repulsion backcrosses), so this χ^2 analysis tests the linkage hypothesis directly, and disposes of it. On an affinity basis, the heterogeneity indicates that some segregations are showing quasi-linkage and others not, or that if all are showing it, some heterozygotes are divergent heterocentrics and some convergent.

As with *Sd-Ca*, the deviation χ^2_1 for *Ca-se* (table 6) is significant (5·9398) but not the heterogeneity χ^2 (8·4640 for 7 d.f.). Rearrangement of the complementary pairs to test linkage shows an insignificant

TABLE 6

Data from the backcrosses—Ca-se

Genotype and reference number of the heterozygote		Segregation of the progeny (genotypes run vertically)					Ratio between complementary pairs	Independence tests		
		Ca	*Ca se*	+	*se*	Total	*Case* & ++ : *Ca* & *se*	χ^2	d.f.	*p*
Ca_{10}/se_4	1a	26	29	33	41	129	62 : 67	0·1938		
	2a	12	12	17	11	52	29 : 23	0·6923		
	2d	32	20	23	35	110	43 : 67	5·2364	1	<0·05
Ca_{10}/se_2	3b	29	30	25	29	113	55 : 58	0·0796		
	4b	35	33	38	40	146	71 : 75	0·1096		
$Case_2/+_E$	5a	21	8	16	11	56	24 : 32	1·1429		
	7b	30	35	37	42	144	72 : 72	0·0000		
Ca_2/sed_4	9a	61	32	48	56	197	80 : 117	6·9492	1	<0·01
Total χ^2								14·4038	8	
Deviation χ^2							436 : 511	5·9398	1	<0·02
Heterogeneity χ^2								8·4640	7	<0·3

departure from independence ($\chi^2_1 = 3·6758$ and the recombination value is 46·88 per cent.). The heterogeneity χ^2 is, as expected, greater, but not quite significant ($\chi^2 = 10·7280$ for 7 d.f.). Thus the data here also strongly favour the affinity rather than the linkage hypothesis.

(ii) *Data from an inbred line*

The control data come from one of the lines used in the outcrosses, Line 2. Segregations of *Sd-Ca* are from animals inbred up to eight generations after the start of the line (table 2B*c* and 2B*d*). Those of *Sd-se* and *Ca-se* come from a later part of the line, owing to the later introduction of *se*, but they are from animals inbred roughly to the same extent (table 2D and E). All are homogeneous and show independence, as expected on an affinity basis.

(iii) *Data from the outcrosses*

Genetically backcross data from genealogical outcrosses provide an additional control. If some unusual viability or chromosomal relation is put forward as a basis for explaining the occurrence of quasi-linkage in the F_1 data, the data from outcrosses, which are genealogically intermediate between them and the inbred, might be expected to show some deviation from independence or heterogeneity.

TABLE 7

Independent backcross segregations

Genotype and number of heterozygotes	Segregations				
	a b	a	b	+	Total
a/Ca (1)	16	16	26	15	73
b/Ca (5)	108	115	122	143	488
b Ca/++ (2)	64	60	53	46	223
b fz/++ (1)	38	34	41	45	158
b/Re (1)	20	25	16	19	80
b/s (1)	7	10	14	20	51
b/Sd (5)	145	176	153	138	612
b Sd/++ (3)	65	84	72	92	313
b se/++ (1)	33	38	47	37	155
b/se (1)	26	26	24	23	99
Ca/fz (1)	37	32	42	47	158
Ca/ln (1)	22	26	20	26	94
Ca s/++ (3)	67	74	65	91	297
fz se/++ (1)	42	36	39	39	156
ln/s (1)	9	12	16	16	53
ln/Sd (1)	10	11	12	20	53
ln/se (1)	5	13	12	20	50
s se/++ (1)	10	15	7	18	50
s/se (1)	41	47	44	64	196
Sd/Re (2)	83	73	89	80	325

Under "segregations", a stands for the mutant placed first in the heterozygote, b for the next, *e.g.* for *a/Ca*, a = *a*, b = *Ca*.

s is probably irregularly penetrant.

se stands for *se*, *d* or both; there were no *se-d* crossovers.

The pairs involving *Ca* have no confirmation of independence from translocation work (Carter *et al.*, 1956, and Slizynski, 1957); the above data are the first for *Ca* with *fz* (Carter and Falconer, 1952, and Michie, 1955*b*).

The data for *Sd* and *Ca* do not fulfill this expectation. Whereas the data from the backcrosses (table 4) show an overall significant excess of the complementary pair *Sd Ca*, ++ over the pair *Sd*, *Ca*, the data from the outcrosses (table 3) show neither significant excess nor heterogeneity. (The slight overall excess is entirely due to the 2×10 cross, where *Sd* and *Ca* are in *different* members of each mating.)

(iv) *Data for other factors*

From the F_1 of the outcrosses, none of the other factors, segregating with each other or with *Sd*, *Ca*, *se* and *b*, give any significant departures

from independence. This may well be because, with three or fewer heterozygotes tested for each two-point, there was not sufficient chance of finding double heterocentrics. All the data for each two-point (including those with *b*) are therefore pooled (table 7).

(v) *Conclusions*

The data discussed in sections (i), (ii) and (iii) show that a significantly high number of deviations from independence have occurred in data from the F_1 of outcrosses between unrelated stocks, and that no such deviations have occurred from inbred data or outcross data. This is in accordance with expectation on an affinity basis. Further analysis disposes of linkage, viability disturbance and other chromosomal or viability relations, leaving quasi-linkage, and thus affinity, as the only plausible hypothesis. On this basis, *Sd*, *Ca* and *se* are near their centromeres, as suspected from earlier evidence, the possible centromeric linkage of *b* remaining uncertain. No new centromere-markers are revealed.

4. MAPPING THE CENTROMERES

On the assumption of affinity, various inferences can be drawn from quasi-linkage data about the positions of the centromeres in relation to the markers used. The ultimate test of the theory is the agreement between different bodies of data on this positioning, and its conformity with the accepted concepts of intra-chromosomal linearity and interference. Inferences about the operation of affinity inter-chromosomally must also be consistent. Centrotype notation should, for any one animal, involve two kinds only—*i.e.* the postulation of only two poles; and mapping procedure should not, in general, produce a wide range of separation values—values much less than 30 per cent. would be suspicious on the grounds that, if common, affinity would have been discovered before now.

The data are now examined from this aspect. (Mapping formulæ in addition to those already given (Wallace, 1957*b*, 1958*a*), will be derived where necessary; page references are to the 1958*a* paper.)

(i) *Linkage group V*

From the *W*-V data the following distances were calculated (pp. 242, 243):

heterozygotes	*fi*	C	*Sd*	
female	13·26	9·92		recombination
male	10·74	16·94		values per cent.

The male data, involving 716 progeny, were balanced for *W-fi* and *W-Sd*; in neither sex did a^t (to the left of *fi*) show quasi-linkage (table 8, p. 234 and table 12, p. 236).

Tables 1A and 1B present two new bodies of data for quasi-linkages involving three different chromosomes: III marked by *W*, VI marked by *Ca*, and XIII marked by *Sp*. *Ca* has a very different effect on the phenotype from that of the other two. It would be an extremely remote coincidence if agreement on centromere position in V were obtained by any cause other than the specific inter-chromosomal relations posited by affinity.

The first striking point is that *Sp*, while showing significant quasi-linkage with *fi* and *Sd*, shows none with a^t, as expected.

The most important feature is the agreement in placing the centromere between *fi* and *Sd*. This may be quantitatively appreciated by a comparison of the estimated λ values given by each of the three bodies of data. λ is an expression of the ratio between *fi*-C and *Sd*-C and is estimated (p. 248) from the observed quasi-linkages as follows:

$$\lambda_{Sd\text{-}fi} = \frac{(1-2Sd\text{-C})}{(1-2fi\text{-C})} = \frac{(1-2\text{M-}Sd)}{(1-2\text{M-}fi)} \qquad \text{(i)}$$

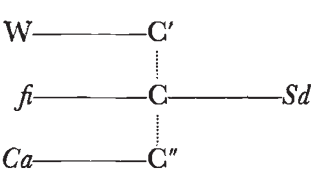
where C is the centromere in V and M is the marker in an independent linkage group. In the male *W*-V data λ is 0·84 with the 5 per cent. fiducial limits at 0·47 and 1·34; in the female data, λ is 1·09—with wider limits but clearly close to the male value. *Ca* and *W* in the female data in table 1A estimate λ jointly as 0·83, a remarkably good agreement. The male and female data for *Sp* in table 1B*b* are pooled as the progeny number for females is small: λ is 1·24, well within the *W*-V fiducial limits.

It may be concluded therefore that all three bodies of data agree closely with a λ value of unity, that is with the centromere almost in the middle of the *fi*-*Sd* segment.

Owing to its size and balance, the *W*-V data are still the most reliable for males; their *fi*-C and *Sd*-C values are therefore the best to use in other affinity calculations for males. The best value for females will be considered in the next section.

(ii) *Linkage groups III and VI*

The data in table 1A show linkage and quasi-linkage relations which may be represented conventionally as follows (see p. 223):



The fact that *W* and *Ca* segregate simultaneously with *fi* and *Sd* gives joint estimates of *fi*-C and *Sd*-C; these, like the single estimates given by *W* in the *W*-V investigation, are independent of variations

in the separation values (p. 223). However, for the singly-marked chromosomes no information can be obtained without the assumption of equal separation values, *i.e.* that C′-C = C-C″ = C′-C″. This assumption has not been tested experimentally: it probably does not introduce a great deal of error, but it should not be ignored when assessing the accuracy of the parameters derived.

The two types of parameter of interest are λ and marker-centromere values.

$\lambda_{W\text{-}Ca}$ is an expression of the ratio between *W*-C′ and *Ca*-C″. This and $\lambda_{W\text{-}Sd}$, $\lambda_{Sd\text{-}Ca}$, etc., are useful for comparison with estimates from any future body of data from which direct comparisons of marker-centromere values are not available. Since marker-centromere values can be obtained here, they will be used in comparisons with others from existing data to assess the consistency of the affinity theory.

$\lambda_{W\text{-}Ca}$ is derived as follows. Assuming equal separation values, there are two formulæ of the form of (i). A joint estimate is obtained from their product:

$$(\lambda_{W\text{-}Ca})^2 = \left\{\frac{(1-2W\text{-C}')}{(1-2Ca\text{-C}'')}\right\}^2 = \left(\frac{1-2Sd\text{-}W}{1-2Sd\text{-}Ca}\right)\left(\frac{1-2fi\text{-}W}{1-2fi\text{-}Ca}\right). \quad . \quad \text{(ii)}$$

Substituting the observed quasi-linkage values of table 1A, $\lambda_{W\text{-}Ca}$ becomes 1·5. $\lambda_{W\text{-}Sd}$ and $\lambda_{Sd\text{-}Ca}$ (using their single formulæ of form (i)) are estimated as 1·39 and 0·69.

The formula for $\lambda_{W\text{-}Sd}$ can be rearranged as follows: $(1-2W\text{-C}') = (1-2Ca\text{-}W)(1-2Sd\text{-C})/(1-2Sd\text{-}Ca)$. With *fi* in place of *Sd*, $(1-2W\text{-C}') = (1-2Ca\text{-}W)(1-2fi\text{-C})/(1-2fi\text{-}Ca)$. Since $(1-2fi\text{-C})(1-2Sd\text{-C}) = (1-2fi\text{-}Sd)$, a joint estimate from their product is given by

$$(1-2W\text{-C}')^2 = \frac{(1-2Ca\text{-}W)^2(1-2fi\text{-}Sd)}{(1-2Sd\text{-}Ca)(1-2fi\text{-}Ca)}. \quad . \quad . \quad \text{(iii)}$$

A similar formula for $(1-2Ca\text{-C}'')^2$ is obtained by transposing *Ca* and *W* throughout. Substituting the observed quasi-linkage values of table 1A, and the *fi-Sd* female value (20·5454) derived from the balanced three-point backcross for V (Wallace, 1957*a*), these estimates become:

$$W\text{-C}' = 7{\cdot}53 \text{ per cent.}, \qquad Ca\text{-C}'' = 17{\cdot}23 \text{ per cent.}$$

The only other value for *W*-C′ available is 12·5 per cent. This is the 1/8 used in the *W*-V calculations to predict the frequencies of various centrotypes in the three generations of that programme, predictions strongly supported by the results (p. 225 and pp. 237-239). An "intelligent guess" by Sir Ronald Fisher, 12·5 per cent. must then have been of the right order of magnitude. The present estimate (from females) is reasonably close to it.

The estimate for *Ca*-C′ will be compared with that in the next section.

It remains to obtain *fi*-C and *Sd*-C. Formula (6) (p. 223), with a further one derived from it by substituting *Ca* for *W*, gives a joint formula from the product of the two. Substitution of the observed quasi-linkage values and the female *fi-Sd* value give

$$fi\text{-C} = 7{\cdot}96 \text{ per cent.}, \qquad Sd\text{-C} = 14{\cdot}97 \text{ per cent.}$$

These joint estimates are preferable to the single estimate from the *W*-V female data. It is worth noting that, in placing the centromere nearer *fi* than *Sd*, they also agree better with the *W*-V male data.

(iii) *Linkage groups II and VI*

Since the two-point data for *Sd*, *Ca* and *se* (tables 4, 5, 6) on the whole show quasi-linkage, some of the animals heterozygous for all three may be triply heterocentric. As these will give further information for mapping the centromeres, it is worth discovering which they are.

This is not at once apparent, for some may be heterocentric for one or two chromosomes, but not all three—as indeed the heterogeneity and deviation χ^2 values suggest. The data must therefore be selected on the individual χ^2 values. Here there is the difficulty of bias in favour of closer quasi-linkage for one pair of factors than for another. This is minimised by selecting as triply heterocentric those animals for which *any two* of the three two-point segregations have a significant independence χ^2, and by considering the *joint* segregation of the three factors (not the segregations in the tables, for which different two-points have different totals). If bias remains, it will tend to equalise the two-point quasi-linkage values (and thus the centromere-marker values) and to underestimate each centromere-marker value.

Two animals only pass the above test, animals 9a and 2d; their joint segregations are given in table 8A. As a test of affinity, their quasi-linkage relations are first examined to see whether a consistent centromere notation can be used on the basis of two types only. Such notation would not be consistent if, from the phase of quasi-linkage for any two of the three two-points, the centrotypes of the three pairs of centromeres were deduced, and it were then found that the centromeric phase of the third two-point, so derived, does not predict exactly the phase of quasi-linkage between its markers. On the basis of their two significant two-point segregations, the centromeres of 9a and 2d can be written respectively as:

$$\frac{Sd\alpha}{+\beta}\ \frac{+\beta}{Ca\alpha}\ \frac{sed\beta}{+\alpha} \quad \text{and} \quad \frac{Sd\beta}{+\alpha}\ \frac{+\beta}{Ca\alpha}\ \frac{sed\beta}{+\alpha}.$$

For 9a, the derived centromeric phase is: divergent (*Sd-se*) and the quasi-linkage is a reversal (56 per cent.), as expected. For 2d, the derived phase is convergent (*Sd-Ca*) and the quasi-linkage is less than 50 per cent. (45 per cent.), also as expected.

TABLE 8

Simultaneous segregations from two triply heterocentric heterozygotes

Heterozygotes (males)	(*P*)	(*Sd*)	(*Ca*)	(*se*)	Total	Recombination per cent.	Independence tests	
							χ_1^2	*p*
A : Individual segregations								
9a : *Sd sed*$_4$/*Ca*$_2$	18 26	35 38	17 24	24 15	197	*Sd-Ca* 57·87 *Sd-sed* 56·85 *Ca-sed* 40·61	4·8782 3·7005 6·9492	<0·05 <0·1 <0·01
2d : *Sd sed*$_4$/*Ca*$_{10}$	22 19	13 13	13 11	12 7	110	*Sd-Ca* 45·45 *Sd-sed* 40·91 *Ca-sed* 39·09	0·9091 3·6364 5·2364	<0·5 ≐0·05 <0·05
B : Combined segregations								
Heterozygotes	(*P*)	(*Sd*)	(*Ca*)	(*se*)	Total	Recombination per cent.		
						Sd-Ca	*Sd-sed*	*Ca-sed*
9a 2d	73 41	44 26	39 24	41 19	197 110	43·32	42·35	40·07
Totals	114	70	63	60	307			

In section B, the headings (*P*), (*Sd*), (*Ca*), (*se*) pertain to the data rearranged so that all recombination values in section A exceeding 50 per cent. $(1-y)$ are now <50 per cent. (y).

When the data of the partially divergent heterocentric are rearranged so that all two-point values are less than 50 per cent. (p. 224), it is seen that the two bodies of data are homogeneous (table 8B), and they agree in showing a smaller quasi-linkage value for *Ca-se* than for the other two-points.

These data are less informative than those considered in the previous section, for no chromosome is doubly marked. The only parameters which can be estimated are the three λ values, and these only on the assumption of equal separation values. If one value is used from another body of data, the others can then be derived, but only on this assumption and the estimates are single, not joint.

The three λ values are estimated as follows:

$$\lambda_{Ca\text{-}se} = 0{\cdot}87 \qquad \lambda_{Sd\text{-}Ca} = 0{\cdot}77 \qquad \lambda_{Sd\text{-}se} = 0{\cdot}67.$$

Confidence limits will be wide because of the relatively small progeny number and loose quasi-linkages (*e.g.* the 5 per cent. limits (p. 248) for $\lambda_{Sd\text{-}se}$ are 1·71 and 0·11). It is striking, however, that the estimate of $\lambda_{Sd\text{-}Ca}$ is very close to that obtained in the previous section, 0·69.

The *Sd*-C male value(16·94 per cent.), used in the above expressions, gives the values

$$se\text{-C}''' = 0{\cdot}81 \text{ per cent.} \quad \text{and} \quad Ca\text{-C}'' = 7{\cdot}09 \text{ per cent.}$$

Confidence limits will be correspondingly wide, but certain comparisons are worth making, taking these quantites as they stand. The *se*-C''' value is compatible with previous evidence (Michie, 1953, 1955*a*, *b*); Michie in fact concludes that *se* is " effectively contiguous with its centromere ". The *Ca*-C value, here for males, is lower than the female value 17·23 per cent., but, as mentioned, it may have been underestimated. It may be said that there is not striking disagreement. With the probable value at 17·23 per cent. or a little less, and the following recombination values (per cent.) for the whole group (Mallyon, unpublished)

females	males
$N-0{\cdot}495-Ca-3{\cdot}797-bt,$	$N-2{\cdot}111-Ca-10{\cdot}247-bt,$

it appears that the centromere is probably outside the group. Data involving a doubly-marked chromosome VI are now accumulating.

There is a final point of interest pertaining to the *Sd*-C value. If the value accruing from the fitted map from the *W*-V data, 12·865 per cent. (p. 247), is used in the *Sd-Ca-se* data, the estimate for *se*-C''' becomes negative. This map is based on the assumption of the $\frac{1}{4}\chi^2_4$ metric (Owen, 1949, 1950), and the fit is not perfect ($\chi^2 = 4{\cdot}5009$ for 1 d.f.). A value of *Sd*-C chosen to agree better would have to be less than this, and so to give, here, a more impossible value to *se*-C'''. Moreover, the improved female data (table 1A) give an *Sd*-C value which would also fit such a map less well than do the *W*-V female data which prompted the idea of fitting. There are also multi-point linkage data which suggest that in mice interference is stronger than this metric indicates (Parsons, 1958). Hence, although the affinity data do not completely contradict the applicability of the $\frac{1}{4}\chi^2_4$ metric, they do indicate that the exact centromere-marker value derived from affinity data, rather than one fitted to a particular metric, should be used in affinity work—at least until more is known about interference.

(iv) *Separation values*

The *Sd*-C and *fi*-C values from the *W*-V data for females, and the *W*-C′ and *Ca*-C″ values so far obtained (section ii) may in turn be used to derive an estimate of the average of the separation values in table 1A. These involve C, C′, C″ (*i.e.* the centromeres of groups V, III and VI). Following the form of formula 10 (p. 224), the appropriate equation is

$$(1-2 \text{ av. sep.})^6 = \frac{1-2Sd\text{-}W)(1-2Sd\text{-}Ca)(1-2Ca\text{-}W)^2(1-2fi\text{-}W)(1-2fi\text{-}Ca)}{(1-2W\text{-C}')^4(1-2Ca\text{-C}'')^4(1-2fi\text{-}Sd)^2} \quad . \quad \text{(iv)}$$

whence the average separation value = 38·22 per cent.

The C′-C value obtainable for the *W*-V data for males, with the same *W*-C′ value (there being no estimate for it for males), is given by

$$(1-2\text{C}'\text{-C})^2 = \frac{(1-2W\text{-}fi)(1-2W\text{-}Sd)}{(1-2fi\text{-}Sd)(1-2W\text{-C}')^2} \quad . \quad . \quad \text{(v)}$$

whence C′-C = 38·07 per cent.

Finally, the value for the same two centromeres in the data for males of table 8 can be obtained. Here the *Sd*-C value for males from *W*-V, and the derived *se*-C‴ value form the denominator in the equation

$$(1-2\text{C}'\text{-C}) = \frac{(1-2Sd\text{-}se)}{(1-2Sd\text{-C})(1-2se\text{-C}''')} \quad . \quad . \quad \text{(vi)}$$

whence C′-C = 38·24 per cent.

These three estimates are strikingly close. They are not entirely independent since certain values, as shown above, are used in more than one of them. However, that their agreement is real and not merely systematic is readily seen from the fact that the numerators of all the expressions of estimation contain only independent observational values. 38 per cent. seems then a reasonable working figure to use for future data from laboratory stocks when direct estimates are not available.

(v) *Conclusions*

The data presented in this paper present no disagreement *inter se*, or with data previously presented, on any of the marker-centromere values obtained. For some bodies of data confidence limits are rather wide, but the estimates are remarkably close (*W*-C′, *Ca*-C″, *se*-C‴, $\lambda_{Sd\text{-}Ca}$); for others, where confidence limits are smaller, all new data are well within these limits (*fi*-C, *Sd*-C). Centrotype notation is consistent in the two cases where this could be tested (two animals triply heterocentric for *Sd*, *Ca* and *se*); and all three bodies of data which can do so, give virtually the same separation value (38 per cent.).

5. SUMMARY

An outcrossing programme in mice, completed in 1954, tests the theory of affinity, and provides information on the identity of centromere-markers and their mapping.

(i) *Experimental test of affinity*

An affinity relation is demonstrated, for any two markers, if

(*a*) outbred heterozygotes give heterogeneous data, some individuals showing reversal; or
(*b*) outbred heterozygotes give an overall departure from independence (with or without heterogeneity); and
(*c*) all inbred heterozygotes homogeneously give independence.

Four markers were identified as close to their centromeres from summaries of current and published data (by similar criteria to those above) and used in the programme.

Three markers were involved in quasi-linkages and conjointly satisfy conditions (*a*), (*b*) and (*c*) above. Relevant χ^2 tests are significant at levels ·05 and ·01. There is a simple explanation for the failure of the fourth marker to give quasi-linkage.

The test was thus successful.

(ii) *Evidence from mapping*

A critical test of affinity is whether the information on centromere position derivable from this programme and earlier work is consistent. This is found to be so.

Firstly, data are presented in which three independent markers confirm the mapping of the centromere between the two linked markers in the *W*-V programme given elsewhere (Wallace, 1958*a*).

Secondly, a marker-centromere value derived from these data is found to be close to that used successfully to predict the frequencies of various genotypes and centrotypes in the *W*-V programme.

Thirdly, the outcrossing programme yields three centromere-marker values which agree closely with those derived from other bodies of data.

Finally, three estimates of average separation values are almost identical.

(iii) *Chromosomal and separation values*

From published data and the new data, the best recombination estimates are:

linkage group		heterozygotes
V	*fi*-10·74 per cent.-C-16·94 per cent.-*Sd*	male
V	*fi*-7·96 per cent.-C-14·97 per cent.-*Sd*	female
III	*W*-C′ = 7·52 per cent.	female
II	*se*-C‴ = 0·81 per cent.	male
VI	*Ca*-C″ = 17·23 per cent.	female

The latter estimate suggests the centromere is outside group VI.

The three estimated average separation values are: 38·24, 38·07 and 38·22 per cent.

(iv) *Formulæ*

Formulæ are given for obtaining marker-centromere values in the different types of data presented, and their relative reliability is discussed.

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