THE GENETICS OF *PRIMULA SINENSIS*.

III. LINKAGE IN THE DIPLOID.

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(With One Text-figure.)

In a previous paper (de Winton and Haldane, 1933) the authors described 30 factors in the diploid *Primula sinensis* located at 28 loci. Two of these are closely linked or allelomorphic. Of the 27 outstanding loci 15 are here shown to be situated in four linkage groups containing 5, 4, 4 and 2 members. The haploid number of chromosomes is 12. There are 12 factors, besides others which have appeared since 1931 but have not yet been

TABLE I.

Chromosome	Factor	Expression of recessive
I	s	Pin. Long style, low anther, small pollen grain
	b	Red. Co-pigment needed for magenta colour absent in petals
	x	Sterile double. Reproductive organs represented by petals
	g	Red stigma and gynaecium. Petals deeply coloured
	l	Dark stems and leaves
II	p	Peculiar eye. Eye enlarged, petals rolled, enhances action of f^s
	w	"Feeble minded." Small flowers, poor growth
	f^s	Sutton's crimp. Leaves slightly crimped, eye lobed
	f^l	Lee's crimp. Leaves strongly crimped, eye large
	ch	Stellata. Petals unfringed, 5 instead of 10 calyx teeth
III	m	Fertile double. Extra whorl of petals. Anthers exserted
	y	Fern leaf. Leaf large and narrower than normal
	h	Harlequin. Inner petals lighter coloured than the outer
	k	Coral. Coral pink anthocyanin replaces red
IV	o	Oak leaf. Incised, and divided into three lobes
	mp	Maple leaf. Less serrated than normal, subglabrous

fully studied, to be located in 7 chromosomes, if all are independent of those so far located, which is by no means certain.

Our data are those accumulated since 1904 by Bateson and Gregory at Cambridge, and by Bateson and his colleagues and successors at Merton. These are supplemented, as regards the three factors S, B and G, by the results obtained by Altenburg (1916) in New York. Gregory, de Winton and Bateson (1923) had already described the linkages of S, B, G, L and that of F and Ch. The remaining linkages have been detected since their paper was written.

The factors known to be linked with one another are shown in Table I, and their positions on chromosome maps in Fig. 1. Their action

is fully described by de Winton and Haldane (1933). f^s and f^l are allelomorphs. The precise location of w is uncertain.

Constancy of linkage.

The cross-over values of Tables III–VI are given with their standard errors. The latter are calculated on the hypothesis that the differences in linkage values between different families derived from the same type of mating are wholly due to random sampling. Is this true, or in other words are we justified in adding together data from many families, and

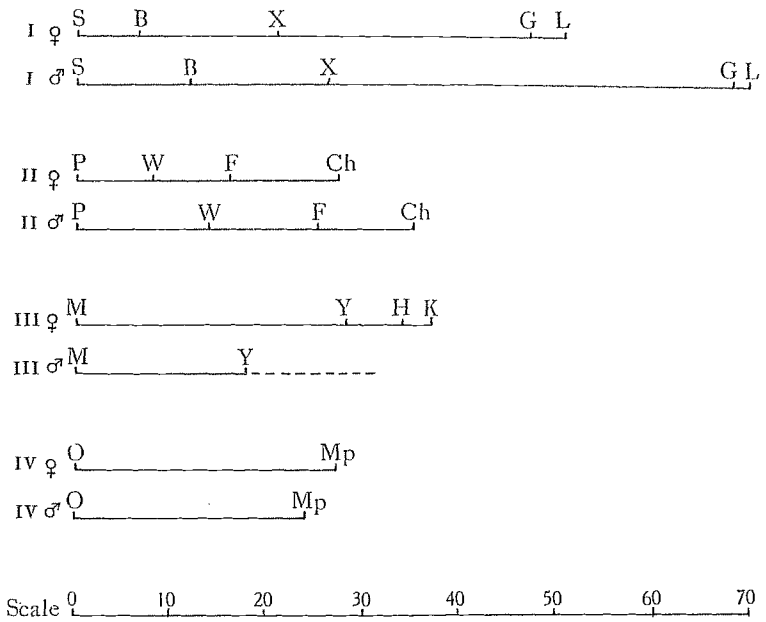

Fig. 1. Provisional linkage map. The position of W, and the relative positions of H and K are uncertain.

treating such data as homogeneous? To answer this question we must discover whether the linkage values in different families diverge more than is to be expected as the result of sampling.

The method by which a group of families is tested for constancy of linkage is given in Appendix I. For each group of families so tested we arrive at Pearson's χ^2 and thence at a number ξ. The different values of ξ, if they are an expression of sampling errors only, should be normally distributed about zero with standard deviation unity.

The calculation is rather tedious, so we confined our examination to the back-cross values for adjacent loci, except that as there were only

two sufficiently large families showing YH, and one showing HK linkage, the YK families are considered. If we included other data, such as SG linkage, the different values of χ^2 would no longer be independent. In the first chromosome, where data are extensive, we only tested families showing coupling, and in the case of Bb Gg, where there were no less than 250 families, we only included families from BGs.bgs.

Finally, since the χ^2 test gives erroneous results when the expectation in the smallest class is less than about 5, we omitted small families. Thus only families of over 250 from GL.gl ♂, over 150 from GL.gl ♀, over 100 from SB.sb ♀, and so on, were included. Provided that the exclusion is based on the total family size this introduces no bias.

The results are assembled in Table II. It will be seen that the overall value of ξ is positive, and only 4 out of its 13 values are negative. The deviation of the mean is 0·27, the standard deviation being 0·28; that is to say that the series as a whole suggests that nothing other than sampling error is affecting linkage. In no series of families does ξ reach the value of 2, which is generally regarded as significant. Moreover, the positive values of ξ are due to a very few families. The largest value, for FCh ♂, is 1·52. If we omitted one family, 14/21, which consisted of 45 **FCh**, 6 **Fch**, 10 **fCh**, 30 **fch**[1], a very large excess of cross-overs, χ^2 would be reduced to 12·58, and ξ to 0·87, while the mean value of ξ would fall to 0·22, which is only just significantly positive. Similarly the removal of one family, 72/28, with a very low cross-over value, would reduce the value of ξ for ChP ♂ from 1·39 to 0·55. Without these two families, the mean value of ξ would be only 0·15, which is not significant.

We conclude that there are probably significant deviations in the linkage value, but that they are confined to very few families, and that the observed deviations are mainly due to sampling. We may put the matter in another way. The total value of χ^2 is 171·0. The total expected value is 156, the number of degrees of freedom, so that $\xi = +0{\cdot}86$, which is not in itself significant. Thus there is an excess of about 9·6 per cent. in the variance above that due to random sampling. Hence the standard errors of the linkage values are slightly too low. They should actually be about 5 per cent. above the values given. Nevertheless the order of magnitude is quite correct.

It is of interest that the cross-over values obtained in this work are far more stable than the single-factor ratios of 1 : 1 to 3 : 1. de Winton and Haldane (1933) found that while in the case of some factors the

[1] Here and throughout heavy letters are used to denote phenotypes. Thus **F** means FF or Ff, **f** means ff.

latter were stable, in others they varied from family to family in such a way that the odds against deviations being due to sampling error were millions to one. The probable explanation is as follows. Factors which upset the ratio of dominants to recessives are common. They are in the same chromosome as the factor under consideration, and either have a lethal or sublethal effect, or alter the relative fitness of gametes at a time of intense competition. Linkage values may be greatly altered by translocations or inversions. But our own back-cross data show no evidence of any such phenomena. The F_2 data, which have not been used for Table II, certainly show a change in the linkage of M and Y between 1907 and 1928.

TABLE II.

Tests for constancy of linkage.

Factors	No. of families	χ^2	ξ
SB ♀	15	21·1	+1·29
SB ♂	16	21·5	+1·16
BG ♀	48	40·7	−0·61
BG ♂	15	11·6	−0·35
GL ♀	4	3·33	+0·40
GL ♂	3	0·173	−1·36
FCh ♀	17	10·4	−1·03
FCh ♂	11	18·5	+1·52
ChP ♀	12	11·5	+0·25
ChP ♂	15	21·8	+1·39
MY ♀	4	2·64	+0·12
YK ♀	4	2·47	+0·04
OMp ♀	5	5·25	+0·64
13	169	171·0	+0·86

Chromosome I.

The first chromosome contains the loci of S, B, X, G, and L in that order. In 1934 further evidence was obtained which makes it almost certain that a sixth factor is located in this chromosome. This new factor as far as we yet know has no effect on G (green stigma) plants. But in gg plants it is a semi-dominant suppressor of the effect of gg on the petals. Thus plants carrying it in the homozygous condition have a red stigma, but petals as light as the corresponding G plants. Its locus appears to be near that of S, very possibly on the other side of S from G.

The linkage data are summarised in Table III. The second to fifth columns give the results from selfings or crossings *inter se* of double heterozygotes. *C* represents coupling, *R* repulsion. In column 7 the mean cross-over value from these data is calculated from Fisher and Balmukand's (1928) table, or directly in the case of G and L. Column 8 gives $\theta = pq$ or $(1-p)(1-q)$, as estimated from these data. Column 9

gives the amount of information about θ. Next follow the data on back-crosses, distinguished according as the heterozygous parent was used as a ♀ or ♂. Here coupling and repulsion data are pooled. In column 13, x and y represent the number of families in Altenburg's data, which have not been published. His data cover 392 plants from $\frac{SBG}{sbg} \times$ ssbbgg and 3292 from ssbbgg $\times \frac{SBG}{sbg}$. He designated S, B and G by the symbols L, R and S. His cross-overs on the male side were SB 12·45, BG 34·57, SG 41·86, agreeing satisfactorily with our values of 11·56, 35·52 and 41·03.

The data involving X are inadequate, and cannot be supplemented until more Xx plants are obtained. xx plants are sterile, and have to be reproduced vegetatively, while unfortunately our Xx stock has been lost. However, three fertile Xx plants were obtained in the past, and their selfing gave the results here tabulated. S and s cannot be distinguished on xx plants. The location of X between the loci of B and G is made clear by families $2^A/19$ and $5^A/19$, from $\frac{BxG}{bXg}$ selfed. They consisted of:

	BXG*	**BXg**	**BxG**	**Bxg**	**bXG**	**bXg**	**bxG**	**bxg**
Observed	35	19	32	1	11	11	1	0
Expected	42·0	14·0	25·7	1·5	14·9	12·3	0·6	0·01

The expectations are calculated on the supposition that the cross-over percentages on the ♀ side are BX 13, XG 23, BG 32, on the ♂ side BX 16, XG 23, BG 35, with 2 per cent. double crossing-over in each case. The agreement is fair. It will be seen that there has been crossing-over between B and X and between X and G in two pairs of gametes which fused, but that there is no evidence of double crossing-over.

The data regarding this chromosome are particularly interesting because the factor pair S, s is the basis of natural heterostylism; and as heterostylism can be compared with sex, though illegitimate unions in this species are fairly fertile, this chromosome may perhaps be compared with the sex chromosomes of dioecious plants and animals. There is, of course, no visible difference between S and s chromosomes. Now Ernst (1933) in the related *Auricula* group of *Primula* species, finds hexamorphic heterostylism, which he attributes to the interaction of three pairs of factors, controlling the length of the style, the position of the anthers and the size of the pollen grains. As no crossing-over has been observed, Brieger (1930), Stern (1930) and Haldane (1933) have interpreted the phenomenon as due to multiple allelomorphism. But it

TABLE III.

Data on linkage in Chromosome I.

Factors	F_2 XY	Xy	xY	xy	No. of families	Mean c.o.v.	θ	I	Sex	B.C. total	B.C. cross-overs	No. of families	Crude c.o.v.	I	Corrected c.o.v.	Standard error
SB *C*	3532	181	214	1187	53	7·74	0·8512	19,140	♀	6481	446	142 + *x*	6·88	76,050	6·25	0·33
SB *R*	58	22	26	0	4	0	0	—	♂	7272	908	69 + *y*	12·49	66,510	11·56	0·35
SX *C*	117	11	37		2	13·85	0·0191	—	—	—	—	—	—	—	13·85	(7·43)
SG *C*	927	234	255	161	20	37·57	0·3898	2,470	♀	6107	2066	112 + *x*	34·39	26,630	34·40	0·59
SG *R*	1639	612	699	139	21	41·19	0·1696	4,822	♂♀	8127	3334	60 + *y*	41·02	33,610	41·03	0·53
SL *C*	1775	442	524	286	22	39·27	0·3688	4,732	♀	2831	1063	46	37·55	12,080	37·53	0·82
SL *R*	878	322	405	70	24	39·70	0·1576	6,769	♂	2533	1051	18	41·49	10,430	41·45	0·87
BX *R*	54	33	22	1	2	18·46	—	—	—	—	—	—	—	—	18·46	(18·28)
BG *C*	4262	1016	997	723	86	35·14	0·4207	10,910	♀	9537	3014	169 + *x*	31·60	44,120	32·37	0·45
BG *R*	1697	592	671	120	34	40·63	0·1650	6,424	♂♀	8710	3021	81 + *y*	34·68	38,450	35·52	0·48
BL *C*	2560	649	690	408	51	38·77	0·3749	6,770	♀	3251	1177	51	36·20	14,070	36·73	0·76
BL *R*	975	360	414	70	19	35·98	0·1295	4,773	♂	2467	892	23	36·16	10,680	36·86	0·84
XG *R*	88	46	50	3	3	22·46	—	—	—	—	—	—	—	—	22·46	(19·41)
XL *R*	19	3	7	0	1	0	—	—	—	—	—	—	—	—	0	—
GL *C*	977	16	19	360	24	2·491	0·9508	14,420	♀	3146	115	60	3·66	89,310	3·61	0·31
GL *R*	3513	1625	1631	3	70	4·39	0·001929	881,800	♂	3024	56	37	1·85	166,300	1·82	0·23

is unusual for a series of allelomorphs to control such disparate morphological features independently. If Ernst's hypothesis is correct S is really a group of three or more dominant factors. Only a few plants of *P. sinensis* have been examined for size of pollen grains, but no case of the dissociation of the supposed factors for style length and anther position has been observed in over 11,000 plants from back-crosses, and 7000 from F_2's, in which crossing-over might have occurred, and would probably have been detected by the production of homostylism. Hence if S is a group of factors they are probably held together as the result of a localised difference between the S and s chromosomes, *e.g.* an inversion. If this were so, other factors in the same chromosome should exhibit differences of linkage intensity in heterozygotes according as they are Ss or ss (few SS plants have been tested). The difference in linkage between SsBbGg and ssBbGg plants is not significant. But if the new factor is on the opposite side of S from B, it should be quite possible to detect the effects to be expected on Ernst's theory, and experiments to test it are being undertaken.

CHROMOSOME II.

This chromosome contains the loci of P, W, F and Ch, probably in that order, though the position of W is uncertain. The data for linkage calculation are given in Table IV. The data regarding F are mainly for segregation between the most extreme allelomorphs, F (flat) and f^1 (Lee's crimp), however the data where the intermediate allelomorph f^s (Sutton's crimp) is segregating are concordant. The largest group is from $\frac{f^s\ Ch}{f^1\ ch}$ selfed, which gave 508 f^s **Ch**, 27 f^s **ch**, 44 f^1 **Ch**, 156 f^1 **ch**, giving a mean cross-over value of 9·75 per cent.

The locus of W is about 20 units distant from that of Ch. If the order were F Ch W, the mean cross-over value between F and W would be about 30 units, and we should expect to find 1·9 wwff from $\frac{Wf}{wF}$ selfed, whereas none were obtained. Hence it is probable, though by no means certain, that the loci of W and F are near together, and as the linkage between Ch and W is somewhat more intense than that between Ch and P, but less so than that between Ch and F, the order is probably P W F Ch. We are at present engaged in building up a stock in which w, f and ch are coupled. It will however be very difficult to study the linkage of p and w, since on most w plants p cannot be scored, and F and Ch may affect the possibility of scoring p in such cases.

Chromosome III.

This chromosome contains the loci of the factors M, Y, H and K, probably in that order, and possibly that of R, the order being M Y H K R. The study of linkage has been made difficult by three facts. In the first place the factor h (harlequin) has no visible effect on kk (coral) plants, except in rare cases when it causes a diminished size of the outer petals. Hence in a back-cross only the Kk plants are available for linkage data. Our hhkk plants are derived from the self-fertilisation of a harlequin plant in an F_2 which proved to be Kk. The crosses Hk.hK × hhkk and reciprocally has given 16 **HK**, 279 **hK**, 398 **k**. Similarly the difference between **R** and **rr** is not always apparent on **kk** plants. **kR** petals are much more deeply coloured than **kr**, other things being equal. But B and I also dilute the colour of coral petals, hence if either of these (or possibly other) factors are segregating, r cannot be scored. KR.kr × kkrr has given: 130 **KR**, 77 **Kr**, 92 **kR**, 115 **kr**, corresponding to $40{\cdot}72 \pm 2{\cdot}41$ per cent. crossing-over, while Hr.hR selfed has given: 168 **HR**, 63 **Hr**, 42 **hR**, 6 **hr**, corresponding to $36{\cdot}60 \pm 5{\cdot}11$ per cent., and M and Y show no linkage with R.

The linkage of M and Y was not discovered earlier for the following reasons. In 1906 Gregory selfed four My.mY plants, obtaining:

188 **MY**, 39 **My**, 62 **mY**, 33 **my**.

These figures deviate from independence in the direction indicating coupling rather than repulsion, whereas the corresponding figures from 1928 onwards, which alone are given in Table V, agree fairly well with those derived from other crosses. It remains to explain the complete divergence between Gregory's results and our own. Gregory's notes are carefully made, and the morphological character of his doubles was the same as that of our own. Our ferns are descended from his stock, but our doubles are not, so we may be dealing with different factors. On the other hand it would be rather surprising if two different recessive factors not only gave an extra whorl of petals, but caused exsertion of the anthers in pin plants. The most likely hypothesis is that owing to a translocation the factor M in Gregory's plants lay in a different chromosome to Y. Segmental interchange has not yet been detected in *Primula sinensis*, but this is not surprising, as semi-sterility would not be detected unless pollen and developing ovaries were specially examined.

The data for linkage of M, Y, H and K are given in Table V. The order of M, Y and H is clear from experiments where all three are segregating. The provisional order for H and K is based on their linkage

TABLE IV.

Linkage data for Chromosome II.

Factors		F_2 XY	Xy	xY	xy	No. of families	Mean c.o.v.	θ	I	Sex	B.C. total	B.C. cross-overs	No. of families	Crude c.o.v.	I	Corrected c.o.v.	Standard error
PF	*C*	600	58	90	109	9	20·34	0·6346	1,610	♀	1253	182	6	14·52	10,090	15·10	0·95
PF	*R*	301	112	146	11	4	30·68	0·09523	1,698	♂♀	1079	239	8	21·32	6,256	22·15	1·16
PCh	*C*	1149	172	260	162	16	31·36	0·4711	1,379	♀♂	2030	469	13	23·10	11,430	23·92	0·90
PCh	*R*	369	124	165	15	6	32·15	0·1033	2,085	♂♀	1815	515	15	28·38	8,933	29·45	1·01
FCh	*C*	2972	171	190	831	58	9·18	0·8249	11,250	♀♂	2613	283	25	10·83	22,920	10·35	0·55
FCh	*R*	773	407	309	3	15	9·51	0·009044	41,200	♂	1723	165	23	9·58	19,890	9·04	0·58
PW	*R*	92	40	42		3	—	S.E.									
WF	*R*	82	32	22	0	3	—	—									
WCh	*C*	232	23	13	28	6	16·09	2·37									
WCh	*R*	450	138	107	6	10	27·61	3·45									

TABLE V.

Factors		F_2 XY	Xy	xY	xy	No. of families	Mean c.o.v.	θ	I	Sex	B.C. total	B.C. cross-overs	No. of families	Crude c.o.v.	I	Corrected c.o.v.	Standard error
MY	*C*	529	68	41	54	5	22·09	0·6070	1232	♀	389	89	5	22·88	2356	24·36	1·74
MY	*R*	329	164	102	8	6	25·77	0·06692	2650	♂♀	284	42	2	14·79	2254	16·49	1·79
MH		—	—	—	—	—	—	—	—	♀♂	273	76	3	27·84	—	—	2·71
MH	*R*	526	185	199	21	10	33·47	—	—	—	—	—	—	—	—	—	—
MK		—	—	—	—	—	—	—	—	♀	286	92	3	32·17	—	—	2·76
MK	*R*	357	155	56	4	6	26·26	—	—	—	—	—	—	—	—	—	—
YH		—	—	—	—	—	—	—	—	♀	273	21	3	7·69	—	—	1·61
YH	*R*	318	134	99	0	6	0	—	—	—	—	—	—	—	—	—	—
YK		—	—	—	—	—	—	—	—	♀	1351	103	4	7·62	—	—	0·72
YK	*R*	196	102	114	1	4	9·09	—	—	—	—	—	—	—	—	—	—
HK		—	—	36		—	—	—	—	♀♂	262	14	3	5·34	—	—	1·39
HK	*R*	87	33			4	—	—	—		33	2	1	6·06	—	—	4·15

values with M. Those with Y are inconclusive on this point. A stock recessive for all four factors is in preparation.

Chromosome IV.

This contains the loci of O and Mp. The back-cross values are given in Table VI. The only F_2 data are of six families from Omp.oMp selfed, which gave:

205 **OMp**, 62 **Omp**, 96 **OMp**, 10 **omp**,

corresponding to a cross-over value of 12·8 per cent. with a large standard error. No satisfactory correction can be made, but it is likely that the true values are slightly lower.

TABLE VI.

Factors	Sex	B.C. total	Cross-overs	No. of families	C.O.V.	S.E.
OMp	♀	479	114	5	23·80	1·95
OMp	♂	646	136	5	21·53	1·60

Allelomorphism or close linkage of D and E.

D (dominant white) and e (recessive flake) are very closely linked. If they are allelomorphic only three types of gamete, DE, dE and de, should exist. Now from the cross ddee × DE.de we should expect to find a majority of DE.de (white) and de.de (flake), and crossing-over would give dE.de (fully coloured) and De.de in about equal numbers. The phenotypic appearance of the latter is of course conjectural. Most of our work was done with gg plants, on which DD**E** and Dd**E** both have a certain amount of pigment, and the flakes of ddee are numerous. Ddee was expected to be a flaked plant with rather pale flakes confined to the inner part of the corolla, since Dd**E**gg plants have a white edge. No plants of this kind have been observed with certainty, but they might be indistinguishable from the rather pale flakes which occur among ddeegg plants.

The following are the results obtained by crossing DDEE (white or "Duchess") with ddee (flaked) and selfing the F_1 or back-crossing it to ddee:

	DD and Dd	dd**E**	ddee
DE.de selfed	844	4	238
DE.de × de.de	17	0	31
de.de × DE.de	155	1	152

At first sight the five dd**E** (fully coloured) plants would seem to be due to crossing-over. But this is not necessarily so. The factor e occasionally mutates to E. The mutation frequency is 0·39 per cent. On this

basis we should only expect about 2·2 whole coloured plants in the F_2. As the frequency of back-mutation of e varies in different lines the presence of four plants is no evidence of crossing-over.

It is not proposed to attempt further work on this problem till other factors linked with D and E have been found. If some of the exceptional plants are due to crossing-over they should be found associated with crossing-over involving these other factors.

Whites allelomorphic with recessive flakes are found in *Antirrhinum* (Wheldale, 1909), *Lathyrus* (Punnett, 1932), *Pisum* (de Haan, 1930) and *Portulaca* (Ikeno, 1929). In all these cases, however, the white was recessive. Our white is fully dominant in G diploids, incompletely so in gg diploids and all tetraploids. So the cases are not quite parallel. If the three factors are allelomorphic they could be designated as E^D, E and e. E^D, or D, is a dominant white, and e a recessive white with a strong tendency to back-mutation, both somatic and germinal. The presence of dominant and recessive allelomorphs at the same locus is known in *Drosophila*, *e.g.* dominant and recessive eyeless. The white parts of white and flaked flowers agree in containing about the same amount of flavone, determined by the factor B.

Independence of the linkage groups.

There is little doubt of the independence of these four from one another. Table VII gives some representative "cross-over values" from back-cross data only. In the event of independence they should be

TABLE VII.

Factors	Total	Cross-overs	Apparent c.o.v. %	σ %	D ÷ σ
SP	664	309	45·03	1·94	2·51
SCh	966	475	49·17	1·61	0·51
BM	1089	527	48·39	1·52	1·06
BY	271	141	52·03	3·04	0·67
LP	664	322	48·49	1·94	0·78
GCh	5548	2792	50·32	0·67	0·48
ChM	282	131	46·45	2·98	1·19

50 per cent. Male and female data are grouped together. The values for S and P suggest linkage, however, the coupling F_2 data give a mean c.o.v. of $51·05 \pm 2·24$, and the repulsion data give a value of $55·02 \pm 4·59$. So linkage is improbable. Similarly on the basis of F_2 data S, G seems to be independent of O, Mp, and so on, though apparent cross-over values of about 45 per cent. are sometimes found. But these are generally inconsistent with other data.

INDEPENDENCE OF OTHER FACTORS.

Since the publication of our last paper the two characters, "cup" leaf and "claw" leaf, have been definitely shown to be due to recessive factors; the relation of Q and U is still not clear. Two new characters have appeared, one being a dominant and the other a recessive. Including these two we have 14 loci outside the linkage groups, those of:

R (blue petal colour).
D, E (white and flake).
A′, a (no eye and large eye).
J′ (green stem, "Sirdar" petals).
N (leafy bracts).
T (tongue leaf).
Iv (ivy leaf, extinct).
C (cup leaf).
I (intense petal colour).
Q (rosetted habit).
Z (claw leaf).
Pi (pistilloid stamens, undescribed recessive).
Ye (yellow leaf, undescribed dominant).

Of these R is very likely in Chromosome III. Of the rest Iv was not strongly linked with the factors in Chromosomes I, II or III, nor with A, E or V. Nothing more can be said about it. The following seem to be independent of the established groups, and of one another:

(D, E), (A′, a), J, N, T, V.

For example, V and S were at one time suspected of linkage, but the pooled back-cross data gave $50{\cdot}07 \pm 1{\cdot}36$ per cent. crossing-over. The linkages of the remaining six factors have been less thoroughly investigated, those of the last two hardly at all, but no evidence of linkage exists.

We thus have 10 linkage groups marked by independent factors, corresponding to 10 of the 12 chromosomes. While our data are entirely consistent with the chromosome theory of linkage, a few more years will be required before we can definitely point to 12 independent linkage groups as well established as the 10 of maize.

We have decided not to publish the complete data involving 510 types of crossing and selfing of 232 pairs of factors, by which the independence of the various factors is demonstrated, since similar data exist in the literature in large numbers, and the plant is not being systematically studied by other workers.

MULTIPLE LINKAGE.

Three-factor crosses are recorded for SBG, SBL, SGL, BGL, PFCh, and MYH, and four-factor crosses for SBGL. The data involving X have already been presented. Back-cross data are given in Table VIII. They are not independent, since for example 77 per cent. of the data for $\frac{SBL}{s\,b\,l} \times \frac{sbl}{sbl}$ are derived from the cross of $\frac{SBGL}{s\,b\,g\,l}$, as are 23·9 per cent. of the data for $\frac{SBG}{s\,b\,g} \times \frac{sbg}{sbg}$. As there is little crossing-over between G and L the majority of SBL double cross-overs also appear as SBG double cross-overs.

TABLE VIII.

Linkage of SBG (*including Altenburg's data*).

♀ *side* (79 + x *families*).

$\frac{SBG}{s\,b\,g} \times \frac{sbg}{sbg}$	$\frac{S\,b\,g}{s\,B\,G} \times \frac{sbg}{sbg}$	
1673 **SBG**	1 **Sbg**	3344 ——
1654 **sbg**	16 **sBG**	
127 **Sbg**	4 **SBG**	256 +—
125 **sBG**	0 **sbg**	
710 **SBg**	5 **SbG**	1504 ——+
783 **sbG**	6 **sBg**	
42 **SbG**	1 **SBg**	88 +—+
45 **sBg**	0 **sbG**	

♂ *side* (52 + y *families*).

$\frac{sbg}{s\,b\,g} \times \frac{SBG}{s\,b\,g}$	$\frac{sbg}{sbg} \times \frac{S\,b\,g}{s\,B\,G}$	
1999 **SBG**	218 **Sbg**	4287 ——
1864 **sbg**	206 **sBG**	
306 **Sbg**	44 **SBG**	721 +—
351 **sBG**	20 **sbg**	
1149 **SBg**	115 **SbG**	2403 ——+
1033 **sbG**	106 **sBg**	
97 **SbG**	19 **SBg**	235 +—+
108 **sBg**	11 **sbG**	

Linkage of SBL.

♀ *side*, 20 *families*.

$\frac{SBL}{s\,b\,l} \times \frac{sbl}{sbl}$	$\frac{SB\,l}{s\,b\,L} \times \frac{sbl}{sbl}$	
457 **SBL**	50 **SBl**	1033 ——
469 **sbl**	57 **sbL**	
38 **Sbl**	3 **SbL**	90 +—
45 **sBL**	4 **sBl**	
256 **SBl**	21 **SBL**	587 ——+
284 **sbL**	26 **sbl**	
11 **SbL**	1 **Sbl**	33 +—+
20 **sBl**	1 **sBL**	

TABLE VIII (*cont.*)

♂ *side*, 10 *families*.

$\frac{sbl}{sbl} \times \frac{SBL}{sbl}$	$\frac{sbl}{sbl} \times \frac{SBl}{sbL}$		
532 **SBL**	10 **SBl**	1099	——
547 **sbl**	10 **sbL**		
81 **Sbl**	2 **SbL**	181	+——
96 **sBL**	2 **sBl**		
317 **SBl**	7 **SBL**	640	——+
311 **sbL**	5 **sbl**		
32 **SbL**	1 **Sbl**	66	+—+
32 **sBl**	1 **sBL**		

Linkage of SGL.

♀ *side*, 16 *families*.

$\frac{SGL}{sgl} \times \frac{sgl}{sgl}$	$\frac{SGl}{sgL} \times \frac{sgl}{sgl}$		
421 **SGL**	37 **SGl**	917	——
412 **sgl**	47 **sgL**		
220 **Sgl**	16 **SgL**	524	+——
264 **sGL**	22 **sGl**		
21 **SGl**	3 **SGL**	48	——+
21 **sgL**	3 **sgl**		
5 **SgL**	0 **Sgl**	14	+—+
7 **sGl**	2 **sGL**		

♂ *side*, 10 *families*.

$\frac{sgl}{sgl} \times \frac{SGL}{sgl}$	$\frac{sgl}{sgl} \times \frac{SGl}{sgL}$		
641 **SGL**	7 **SGl**	1300	——
645 **sgl**	7 **sgL**		
451 **Sgl**	6 **SgL**	911	+——
449 **sGL**	5 **sGl**		
11 **SGl**	0 **SGL**	24	——+
13 **sGL**	0 **sgl**		
8 **SgL**	1 **Sgl**	13	+—+
4 **sGl**	0 **sGL**		

Linkage of BGL.

♀ *side*, 26 *families*.

$\frac{BGL}{bgl} \times \frac{bgl}{bgl}$	$\frac{BGl}{bgL} \times \frac{bgl}{bgl}$		
582 **BGL**	37 **BGl**	1217	——
551 **bgl**	47 **bgL**		
272 **Bgl**	16 **BgL**	636	+——
326 **bGL**	22 **bGl**		
28 **BGl**	3 **BGL**	58	——+
25 **bgL**	2 **bgl**		
5 **BgL**	1 **Bgl**	14	+—+
6 **bGl**	2 **bGL**		

TABLE VIII (*cont.*).

♂ *side*, 15 *families*.

$\frac{bgl}{bgl} \times \frac{BGL}{bgl}$	$\frac{bgl}{bgl} \times \frac{BGl}{bgL}$	
715 **BGL**	8 **BGl**	1442 ——
711 **bgl**	8 **bgL**	
398 **Bgl**	5 **BgL**	788 +—
381 **bGL**	4 **bGl**	
14 **BGl**	0 **BGL**	30 —+
15 **bgL**	1 **bgl**	
5 **BgL**	0 **Bgl**	9 +—+
4 **bGl**	0 **bGL**	

Linkage of PFCh.

♀ *side*, 6 *families*.

$\frac{PFCh}{pfch} \times \frac{pfch}{pfch}$

475 **PFCh**	947
472 **pfch**	
81 **Pfch**	161
80 **pFCh**	
68 **PFch**	124
56 **pfCh**	
11 **PfCh**	21
10 **pFch**	

♂ *side*, 8 *families*.

$\frac{pfch}{pfch} \times \frac{PFCh}{pfch}$

420 **PFCh**	751	——
331 **pfch**		
103 **Pfch**	225	+—
122 **pFCh**		
41 **PFch**	89	—+
48 **pfCh**		
8 **PfCh**	14	+—+
6 **pFch**		

Linkage of MYH.

♀ *side*, 3 *families*.

$\frac{MYh}{myH} \times \frac{myh}{myh}$

98 **MYh**	194	——
96 **myH**		
29 **MyH**	60	+—
31 **mYh**		
9 **MYH**	16	—+
7 **myh**		
2 **Myh**	5	+—+
3 **mYH**		

Linkage of SBGL.

♀ *side*, 14 *families*.

$\frac{SBGL}{sbgl} \times \frac{sbgl}{sbgl}$	$\frac{SBGl}{sbgL} \times \frac{sbgl}{sbgl}$	
366 **SBGL**	36 **SBGl**	809 ——
361 **sbgl**	46 **sbgL**	
28 **Sbgl**	1 **SbgL**	59 +—
29 **sBGL**	1 **sBGl**	
174 **SBgl**	15 **SBgL**	422 —+—
212 **sbGL**	21 **sbGl**	
19 **SBGl**	3 **SBGL**	41 —+
17 **sbgL**	2 **sbgl**	
4 **SbGL**	1 **SbGl**	16 ++—
10 **sBgl**	1 **sBgL**	
0 **SbgL**	0 **Sbgl**	2 +—+
2 **sBGl**	0 **sBGL**	
2 **SBgL**	0 **SBgl**	8 —++
4 **sbGl**	2 **sbGL**	
1 **SbGl**	0 **SbGL**	2 +++
0 **sBgL**	1 **sBgl**	

TABLE VIII (*cont.*).

Linkage of SBGL.

♂ *side, 9 families.*

$\frac{sbgl}{sbgl} \times \frac{SBGL}{sbgl}$	$\frac{sbgl}{sbgl} \times \frac{SBGl}{sbgL}$	
527 **SBGL**	7 **SBGl**	1084 ——
544 **sbgl**	6 **sbgL**	
80 **Sbgl**	2 **SbgL**	179 -+—
96 **sBGL**	1 **sBGl**	
308 **SBgl**	4 **SBgL**	617 —+—
301 **sbGL**	4 **sbGl**	
9 **SBGl**	0 **SBGL**	19 ——+
10 **sbgL**	0 **sbgl**	
30 **SbGL**	0 **SbGl**	62 -+-+—
31 **sBgl**	1 **sBgL**	
2 **SbgL**	1 **Sbgl**	4 -+—+
1 **sBGl**	0 **sBGL**	
5 **SBgL**	0 **SBgl**	8 —+-+
3 **sbGl**	0 **sbGL**	
1 **SbGl**	0 **SbGL**	1 -+-+
0 **sBgL**	0 **sBgl**	

From these data Table IX has been compiled. The fourth column gives the observed double cross-overs, the fifth the number calculated if there were no interference. The ratio is the coincidence. This ranges from 1·00 to 0·61, with a mean value of 0·78, when the two regions of crossing-over are adjacent, but rises above unity, though not significantly, when they are separated. These results are very similar to those

TABLE IX.

Interference.

Regions and lengths		Sex	Total	Observed doubles	Calculated doubles	Coincidence
SB 6·3	BG 32·1	♀	5192	88	102·4	0·86
SB 11·6	BG 35·0	♂	7646	235	304·8	0·77
SB 6·3	BL 36·7	♀	1743	33	43·8	0·75
SB 11·6	BL 36·9	♂	1986	66	87·6	0·75
SG 34·4	GL 3·6	♀	1503	14	22·2	0·63
SG 41·0	GL 1·8	♂	2248	13	15·2	0·85
BG 32·1	GL 3·6	♀	1925	14	24·3	0·58
BG 35·0	GL 1·8	♂	2269	9	13·7	0·66
PF 15·1	FCh 16·4	♀	1253	21	21·6	0·97
PF 22·2	FCh 7·6	♂	1079	14	22·8	0·61
MY 24·4	YH 7·7	♀	275	5	5·0	1·0
SB 6·3	GL 3·6	♀	1359	4	3·1	1·30
SB 11·6	GL 1·8	♂	1974	5	4·0	1·25

found in *Drosophila*, though interference seems to be somewhat weaker in *Primula sinensis*. It will be seen that, as in *Drosophila*, triple cross-overs occur, three being recorded among 3333 plants.

The data from selfing triple and quadruple heterozygotes are given in Table IV. The calculated values are derived by multiplying the

gametic series deduced from Table VIII. The agreement is on the whole satisfactory, but quantitative tests are rather difficult, since the calculated values are derived from fairly small samples, and have errors of their own. Moreover, no allowance has been made for the deviations in the single factor ratios. In each progeny of a triple heterozygote all members of a certain class are derived from one or two gametes whose factors have undergone double crossing-over. Thus from $\frac{\mathrm{SBG}}{\mathrm{s\,b\,g}}$ selfed all **sBg** plants are either derived from the union of two sBg gametes, or by an sBg and an sbg. Similarly from $\frac{\mathrm{SB\,g}}{\mathrm{s\,b\,G}}$ selfed, all **Sbg** plants are derived from a union of two Sbg gametes, or of an Sbg with an sbg. The plants in these classes are summarised in Table XI. It will be seen that the total is only 80 per cent. of the expectation in the absence of interference. The standard error of the expectation is about 7·4, so the deviation is not significant, but if a correction were made for these plants the coincidence values would be altered by under 2 per cent. on the average.

The data for single crossing-over from F_2 plants have already been incorporated with those from back-crosses. If any **sBgL** plants had been derived from $\frac{\mathrm{SBGL}}{\mathrm{s\,b\,g\,l}}$, or **Sbgl** from $\frac{\mathrm{SB\,g\,L}}{\mathrm{s\,b\,G\,l}}$ they would have been due to triple crossing-over. No such plants were observed, but their joint expectation was only 0·02. So no further data on triple crossing-over can be derived from the F_2 plants.

Linkage differences between female and male sides.

For this purpose only back-cross data are available. They are collected in Table XII. In each case the non-cross-over and cross-over classes of the two sexes were collected in a fourfold table, thus $\frac{a}{c}\Big|\frac{b}{d}$ and the measure of divergence

$$\chi=(ad-bc)\sqrt{\frac{a+b+c+d}{(a+b)(c+d)(a+c)(b+d)}} \quad \text{calculated.}$$

If the two cross-over values are really equal, this has an expected value 0 with normal distribution and standard error of unity. Hence we can calculate the probability P of a divergence as great as that observed, *i.e.* the probability that the apparent sexual difference is due to sampling error. The values of P are given in column 5. The smallest segments are marked with an asterisk, the remainder consisting of several such segments.

TABLE X.

$\frac{SBG}{sbg}$ selfed. 19 families.

	Obs.	Calc.
SBG	1000	995·7
SBg	225	223·3
SbG	32	39·1
Sbg	21	40·2
sBG	55	66·3
sBg	12	13·0
sbG	213	197·2
sbg	173	156·3
	1731	

$\frac{SBg}{sbG}$ selfed. 18 families.

	Obs.	Calc.
SBG	1294	1192·2
SBg	466	535·7
SbG	77	174·3
Sbg	15	11·1
sBG	84	87·9
sBg	27	32·7
sbG	503	458·9
sbg	85	58·3
	2551	

$\frac{SBL}{sbl}$ selfed. 22 families.

	Obs.	Calc.
SBL	1577	1616·6
SBl	408	396·7
SbL	66	70·9
Sbl	34	62·2
sBL	94	111·0
sBl	24	22·2
sbL	409	347·9
sbl	250	234·6
	2862	

$\frac{SBl}{sbL}$ selfed. 16 families.

	Obs.	Calc.
SBL	591	625·6
SBl	225	245·6
SbL	31	20·4
Sbl	2	5·4
sBL	45	40·1
sBl	8	15·5
sbL	246	210·9
sbl	48	32·4
	1196	

$\frac{SGL}{sgl}$ selfed. 3 families.

	Obs.	Calc.
SGL	161	164·8
SGl	3	3·4
SgL	2	2·3
Sgl	45	41·5
sGL	44	43·0
sGl	0	0·6
sgL	1	1·8
sgl	27	25·0
	283	

$\frac{SGl}{sgL}$ selfed. 10 families.

	Obs.	Calc.
SGL	280	198·7
SGl	127	147·1
SgL	105	108·6
Sgl	0	0·05
sGL	98	151·0
sGl	17	27·2
sgL	72	66·0
sgl	0	0·06
	699	

$\frac{Sgl}{sGL}$ selfed. 1 family.

	Obs.	Calc.
SGL	43	55·3
SGl	1	1·1
SgL	1	1·2
Sgl	26	21·0
sGL	31	21·9
sGl	0	0·4
sgL	0	0·2
sgl	3	3·7
	105	

$\frac{SgL}{sGl}$ selfed. 21 families.

	Obs.	Calc.
SGL	985	988·3
SGl	386	381·1
SgL	481	543·4
Sgl	0	0·4
sGL	342	287·8
sGl	245	256·1
sgL	112	93·9
sgl	0	0·03
	2551	

TABLE X (*cont.*).

$\frac{BGL}{bgl}$ selfed. 8 families.

	Obs.	Calc.
BGL	289	289·6
BGl	4	8·0
BgL	2	5·5
Bgl	70	63·2
bGL	70	63·0
bGl	0	0·9
bgL	1	3·4
bgl	46	48·4
	482	

$\frac{BGl}{bgL}$ selfed. 14 families.

	Obs.	Calc.
BGL	616	588·1
BGl	303	327·9
BgL	209	214·9
Bgl	0	0·09
bGL	166	166·2
bGl	50	48·8
bgL	164	161·9
bgl	0	0·15
	1508	

$\frac{BgL}{bGl}$ selfed. 21 families.

	Obs.	Calc.
BGL	1131	1082·3
BGl	419	418·2
BgL	544	623·9
Bgl	0	0·4
bGL	365	334·7
bGl	263	289·6
bgL	111	83·9
bgl	0	0·02
	2833	

$\frac{SBGL}{sbgl}$ selfed. 3 families.

	Obs.	Calc.
SBGL	158	158·7
SBGl	3	3·2
SBgL	2	1·7
SBgl	40	36·1
SbGL	3	6·1
SbGl	0	0·1
SbgL	0	0·3
Sbgl	5	5·8
sBGL	4	10·2
sBGl	0	0·2
sBgL	0	0·2
sBgl	2	1·8
sbGL	40	33·2
sbGl	0	0·4
sbgL	1	1·7
sbgl	25	23·1
	283	

$\frac{SBGl}{sbgL}$ selfed. 9 families.

	Obs.	Calc.
SBGL	261	260·5
SBGl	120	136·3
SBgL	94	79·9
SBgl	0	0·04
SbGL	8	13·4
SbGl	2	1·6
SbgL	8	14·5
Sbgl	0	0·01
sBGL	26	17·9
sBGl	2	7·2
sBgL	4	4·5
sBgl	0	0·01
sbGL	67	60·3
sbGl	15	19·6
sbgL	67	59·0
sbgl	0	0·05
	674	

$\frac{SBgL}{sbGl}$ selfed. 18 families.

	Obs.	Calc.
SBGL	937	913·2
SBGl	357	354·1
SBgL	466	533·7
SBgl	0	0·4
SbGL	48	49·2
SbGl	29	53·6
SbgL	15	9·4
Sbgl	0	0·06
sBGL	62	63·7
sBGl	22	17·8
sBgL	27	30·4
sBgl	0	0·01
sbGL	280	249·8
sbGl	223	212·1
sbgL	85	63·9
sbgl	0	0·01
	2551	

$\frac{PFCh}{pfch}$ selfed. 4 families.

	Obs.	Calc.
PFCh	135	121·2
PFch	6	8·7
PfCh	5	3·0
Pfch	10	13·2
pFCh	10	15·3
pFch	1	1·1
pfCh	6	6·8
pfch	22	25·6
	195	

$\frac{Pfch}{pFCh}$ selfed. 2 families.

	Obs.	Calc.
PFCh	157	140·8
PFch	8	12·1
PfCh	4	14·7
Pfch	46	58·1
pFCh	74	69·9
pFch	5	2·9
pfCh	1	0·4
pfch	6	2·0
	301	

$\frac{PfsCh}{pFch}$ selfed. 1 family.

	Obs.	Calc.
PFCh	62	65·9
PFch	18	18·1
PfCh	34	39·2
Pfch	0	0·4
pFCh	24	16·6
pFch	24	22·5
pfCh	2	1·3
pfch	0	0·008
	164	

$\frac{MYh}{myH}$ selfed. 2 families.

	Obs.
MYH	77
MYh	45
MyH	13
Myh	0
mYH	7
mYh	1
myH	12
myh	0
	155

It will be seen that GL and MY are undoubtedly larger, and SB and PF undoubtedly shorter, on the female than on the male side, while in the other cases of neighbouring loci the evidence is still uncertain. No similar cases are known with certainty in hermaphrodite organisms. Stadler (1926) found slightly more crossing-over on the male than on the female side of *Zea Mays*, but Collins and Kempton (1927) found the opposite in most of their families.

TABLE XI.

F_2 classes showing double crossing-over.

	Obs.	Calc.
sBg	12	17·8
Sbg	15	11·1
sBl	24	31·3
Sbl	2	5·4
sGl	0	0·6
Sgl	0	0·05
sgL	0	0·22
bGl	0	0·9
Bgl	0	0·09
bgl	0	0·02
pFch	1	1·1
pfCh	1	0·4
pfch	0	0·01
	55	69·0

TABLE XII.

Sex differences of linkage.

Factor	♀	♂	χ	P
SB*	6·88	12·48	11·77	3×10^{-36}
SG	34·39	41·02	8·011	6×10^{-16}
SL	37·55	41·49	2·960	0·0031
BG*	31·59	34·68	1·550	0·121
BL	36·20	36·16	0·1047	0·916
GL*	3·66	1·85	4·192	$2·8 \times 10^{-5}$
PF*	14·52	21·32	4·774	$1·8 \times 10^{-6}$
PCh	23·10	28·38	3·740	$1·8 \times 10^{-4}$
FCh*	10·45	9·58	1·351	0·178
MY*	22·88	14·79	3·296	$9·8 \times 10^{-4}$
HK*	5·34	6·06	0·1581	0·874
OMp*	23·80	21·53	1·698	0·89

On the other hand in animals crossing-over is generally more frequent in the homozygous sex (see Stern, 1933), though this is not so in Landauer's (1932) case in poultry.

A possible explanation of our data would be that chiasmata were generally formed further away from the spindle attachment on the female side than on the male, since this attachment is believed to be in the SB region of Chromosome I. But for the present this must remain speculative.

DIFFERENCES BETWEEN COUPLING AND REPULSION.

Theoretically, back-crosses involving coupling and repulsion should give the same linkage values. The results are compared in Table XIII for all cases where over 100 plants were available in each cross. In this connection we have not used Altenburg's data, as they give figures for coupling only, of which plenty are available, whereas repulsion data are

TABLE XIII.

Comparison of coupling and repulsion.

Factors	Coupling c.o.v.	Repulsion c.o.v.	χ	P
SB ♂	12·51	12·72	0·3613	0·74
SG ♂	40·74	38·94	0·9230	0·35
SL ♀	37·82	33·13	1·200	0·23
BG ♀	31·27	45·32	4·957	7×10^{-7}
BG ♂	34·69	37·84	0·6890	0·49
BL ♀	36·53	30·06	1·674	0·094
GL ♀	3·54	4·63	0·9865	0·32
PCh ♀	23·51	19·63	1·277	0·20
PCh ♂	28·11	30·57	0·7157	0·47
FCh ♀	11·23	4·38	2·496	0·013
FCh ♂	9·61	9·31	0·1358	0·89

rather few. In column 4 the measure of divergence χ is given. It will be seen that the linkage is more intense in repulsion than coupling in 6 out of the 11 cases, in fact there is no significant difference between the two. Except in the case of BG ♀ the differences are such as might be expected as the result of sampling. In this case the numbers are small, and the single factor ratios extremely aberrant. The divergence is largely due to one family, 106/12, which accounts for 34 per cent. of the total, and consisted of 33 **BG**, 32 **Bg**, 16 **bG**, 14 **bg**. With this single exception the figures are quite satisfactory, though the FCh ♀ divergence is suspiciously large. In 106/12 the proportion of bb was only 32 per cent., instead of 50 per cent., so some lethal or other similar factor was at work. A lethal in the neighbourhood of S would account for the shortage of bb, and would somewhat raise the cross-over value, but not to the extent observed.

We conclude that the observed differences between coupling and repulsion are not systematic but due to a few aberrant families.

SIMULTANEOUS CROSSING-OVER IN TWO CHROMOSOMES.

In order to see whether there was any correlation between the crossing-over of unlinked factors, plants of composition $\frac{\text{sbgl}}{\text{sbgl}}\,\frac{\text{chp}}{\text{chp}}$ were made up and crossed with a heterozygous dominant. The resulting

$\frac{\text{SBGL ChP}}{\text{s b g l chp}}$ were crossed as females and males with the sextuple recessive, and 664 progeny raised. The results are summarised in Table XIV.

TABLE XIV.

Simultaneous crossing-over in two chromosomes.

	181 and 183/30, ♀				182 and 184/30, ♂				
	ChP	**Chp**	**chP**	**chp**	**ChP**	**Chp**	**chP**	**chp**	Total
SBGL	47	13	22	41	26	8	9	26	192
SBGl	3	2	—	2	—	—	1	1	9
SBgL	—	—	—	—	—	—	—	—	—
SBgl	21	5	13	28	16	3	5	14	105
SbGL	—	—	—	—	1	2	—	2	5
SbGl	—	—	—	—	—	—	—	—	—
SbgL	—	—	—	—	—	—	—	—	—
Sbgl	2	2	—	5	5	3	—	7	24
sBGL	—	—	2	4	4	1	4	5	20
sBGl	1	—	—	—	—	—	—	1	2
sBgL	—	—	—	—	—	—	—	—	—
sBgl	—	—	1	—	2	—	—	—	3
sbGL	22	5	11	34	8	—	2	20	102
sbGl	1	—	—	—	—	—	—	—	1
sbgL	—	—	—	4	1	—	—	—	5
sbgl	46	12	7	55	22	9	11	34	196
		Total 411				Total 253			

TABLE XV.

Independence of crossing-over in separate chromosomes.

		♀		♂	
Factors		Unchanged II	Cross-over II	Unchanged II	Cross-over II
SB, ChP	Unchanged I	304	90	168	48
	Cross-over I	12	5 (3·9)	27	10 (8·5)
BG, ChP	Unchanged I	210	60	132	46
	Cross-over I	106	35 (32·6)	63	12 (17·2)
GL, ChP	Unchanged I	305	93	192	57
	Cross-over I	11	2 (3·0)	3	1 (0·9)

The results are now classified as regards crossing-over affecting each adjacent first chromosome factor pair SB, BG, GL, on the one hand, and Ch and P on the other. The first set of figures in Table XV have the following significance. There were 304 plants in which no crossing-over had occurred between SB or ChP, 12 which had crossed between SB but not ChP, 90 between ChP and not SB, and 5 which had crossed over between both pairs. In the event of complete independence the last number should be $\frac{95 \times 17}{411}$, or 3·9.

It will be seen that the two crossings-over are quite independent. The sum of the observed values for the smallest class is 65, of the calcu-

lated 66·1. A similar independence is found between crossing-over in two chromosomes of *Pisum* (de Winton, unpublished).

THE PROBABLE LOCATION OF A SPINDLE ATTACHMENT.

In diploid organisms spindle fibre attachments can only be located in relation to the loci of the genes, either where tetrads can be analysed, or when segregation is abnormal, as in "equational non-disjunction" or when the *X*-chromosomes are attached. But in tetraploids or triploids normal segregation provides data on this question. Mather (1935) points out that, in a triplex (AAAa) plant, if the dominant A is located near the attachment constriction it will almost always segregate reductionally at the first meiotic division, and very few aa gametes will be produced. If, however (to consider one possibility only), a single chiasma is formed between its locus and the attachment constriction, the first division will be equational for A and a, and one gamete in 24 will be aa. Probably so high a proportion is never reached in practice, though if the locus is very far from the attachment we may expect one gamete in 28 (Haldane, 1930).

The linkage of S, B and G in the tetraploid has been described by de Winton and Haldane (1931). The cross-over values are intermediate between those found on the two sexual sides of the diploid. They are not significantly different on the two sides in the tetraploid.

Several plants have been found which are without much doubt of the composition GGGg, but yet have given a few gg gametes. On the other hand no SSSs or BBBb plant has yet given a recessive gamete.

For example the plant $181^3/28$, known from its ancestry and its behaviour on selfing and crossing to be SBG.$(sbg)_3$, was selfed. One of its **SBG** progeny was $119^1/29$. This plant, on crossing to $(sbg)_4$ as female and male gave: 103 **SBG**, 19 **SBg**, 0 **SbG**, 2 **Sbg**, 3 **sBG**, 0 **sBg**, 11 **sbG**, 6 **sbg**. It was therefore of composition $(SBG)_2.(sbg)_2$. On selfing it gave 30 **SBG**, 1 **SBg**, 1 **sbG**. One of its **SBG** progeny was $54^1/30$. This plant crossed as a female with $(sbg)_4$ gave 95 **SBG**, 1 **SBg**, as a male 117 **SBG**, 1 **SBg**. That is to say 214 gametes contained at least one S and one B, and two were gg. In order to test the composition of this plant, 11 of its **SBG** progeny were crossed as females with $(sbg)_4$. The results are given in Table XVI. It will be seen that for each of the factors S, B and G, 6 plants were duplex and 5 simplex. Now if $54^1/30$ were triplex we should expect that it would give equal numbers of duplex and simplex when crossed with $(sbg)_4$, if duplex it should give 1 duplex : 4 simplex. There can be no doubt that it was triplex, in view of the good agreement

of the results for all three factors. It is not certain whether it was of composition SBG.SBG.SBg.sbG, or $(SBG)_3$.sbg, but the latter seems more likely.

The plant 51³/30 derived from selfing a plant duplex for S, B and G, gave 356 **G**, 4 g when crossed with $(sbg)_4$. It was simplex for S and duplex for B. Analysis of the progeny confirmed these facts as regards S and B. Seven or eight out of twelve were duplex for G, hence the parent was triplex. Similarly 51²/30 (**SBG**) gave 45 **SBG**, 2 **SBg** when crossed with the triple recessive. Five of the **SBG** progeny were analysed; three were duplex, and two doubtful whether duplex or simplex for G. Thus 51²/30 was almost certainly triplex for all three

TABLE XVI.

Analysis of progeny of 54¹/30.

Family (1932)	SBG	SBg	SbG	Sbg	sBG	sBg	sbG	sbg	Composition of parent
182	45	3	1	1	0	0	4	4	SBG.SBG.sbg.sbg
183	48	7	0	1	0	0	10	4	SBG.SBG.sbg.sbg
184	5	4	1	0	0	0	4	2	SBg .sbG .sbg.sbg
185	24	3	1	0	0	1	14	6	SBG.sbG .sbg.sbg
186	11	1	1	0	0	0	1	5	SBG.sbg .sbg.sbg
187	28	11	2	1	0	0	1	7	SBG.SBg .sbg.sbg
188	22	2	0	0	0	0	1	1	SBG.SBG.sbg.sbg
189	38	6	0	0	0	0	5	1	SBG.SBG.sbg.sbg
190	31	6	0	0	0	0	21	4	SBG.sbG .sbg.sbg
191	26	17	0	0	0	0	4	3	SBG.SBg .sbg.sbg
192	14	8	1	1	0	1	7	17	SBG.sbg .sbg.sbg

factors. 51¹/30 (**SBG**) gave 43 **SBG** with the triple recessive, and 8 **SBG** when selfed. But since some of the latter segregated for G it was presumably triplex.

Three other plants whose progeny was not analysed behaved in a similar manner for G. One of them is particularly interesting as being derived from a simplex selfed. This may of course yield a triplex on selfing, since a Gggg plant may give a small proportion of GG gametes, just as a GGGg may give a few gg gametes. In all, the plants certainly triplex for G gave 8 gg gametes out of 664, while those whose progeny were not analysed gave 14 out of 454.

Besides the plants mentioned above, 54²/30, which was duplex for G, was triplex for S and B, as shown by progeny analysis, and gave 146 **SBG**, 27 **SBg** with the triple recessive.

We thus find that while GGGg plants gave 1–2 per cent. of gg gametes, SSSs and BBBb gave no recessive gametes out of 434. We conclude that S and B obey the laws of segregation laid down by Muller (1914) while G gives a fair number of exceptions. Hence the spindle fibre attachment

is probably located in the neighbourhood of S and B, while G is far enough from it to segregate equationally at the first division in over 1 per cent. of meioses.

DISCUSSION.

The available data regarding linkage agree completely with the postulates of the chromosome theory, which has been verified in great detail in several species of *Drosophila* and in *Zea Mays*, and less completely in *Lathyrus odoratus*, while at present the number of apparently independent factors in *Pisum* is too large. However, our data, bearing only on four chromosomes, are not conclusive, and it is only worth discussing the points of novelty which they present.

In the first place the difference in linkage between the two sexual sides has now been found in three chromosomes with certainty, and probably in a fourth.

Interference is considerably less than is usual in *Drosophila*. Thus in the *X*-chromosome white and vermilion give 30·5 per cent. crossing-over, vermilion and sable 10·1 per cent. The case is parallel with G, B and S on the male side where the values are 35·0 and 11·6. But in *Drosophila* the coincidence is 0·52 (Morgan and Bridges, 1916) as compared with 0·77 in *Primula*. Similarly Bridges and Morgan (1923) found a coincidence of 0·64 for sepia, dichaete and spineless, the distances being 14·8 and 13·0. We find 0·97 for P, F and Ch, the distances being 15·1 and 16·4. In this case the spindle fibre attachment is included in the region between dichaete and spineless.

If coincidence is really as low in *Primula* as in *Drosophila*, we must suppose that the spindle fibres are attached near B, F and Y. Even so the coincidence for BGL, whose mean value is 0·61, is surprisingly high. The comparison with *Zea* is still more striking. Here Stern (1933) lists four coincidence values, none exceeding 0·40, where our lowest value is 0·58.

On the other hand Castle (1933) found a coincidence of 0·70 between C, Y and B in the rabbit, the distances being CY 14·4, YB 26·8. This is more in accordance with our values than with those in *Drosophila*. While therefore it is too early to be dogmatic, it seems likely that in some organisms (*Drosophila* and *Zea*) interference is on the whole greater than in others (*Primula* and *Oryctolagus*). The map distances have been calculated on this hypothesis.

The linkage values found in different families are remarkably constant. Equally constant values are found in other organisms where

closely related stocks are used, and temperature and age carefully controlled. Thus Gowen (1919) in Table B lists cross-over values from 16 *Drosophila* females. For the cross-over values in Region 4 we find $n=15$, $\chi^2=9{\cdot}64$, $\xi=-1{\cdot}06$, an extremely good fit. But age, temperature and other causes give divergences which cannot be explained by chance. Hence *Primula* may be suitable for work on crossing-over which could not be undertaken in other organisms, and is certainly more suitable than *Drosophila* and *Zea* for the application of refined statistical methods.

Appendix I.

The technique of testing for goodness of fit, as given by Fisher (1925) can be considerably simplified. Suppose we have $n+1$ families, totalling N individuals, of which Nc are cross-overs. Then Pearson's (1900) measure of divergence

$$\chi^2=\Sigma\frac{(x_r-ca_r)^2}{ca_r}.$$

It is inconvenient to calculate each term in this sum, since this involves multiplying by the fraction c. We therefore choose a simple decimal c' near to c (*e.g.* $c'=0{\cdot}10$ or $0{\cdot}25$). Then it can easily be shown that

$$\chi^2=\frac{1}{c}\left[\Sigma\frac{(x_r-c'a_r)^2}{a_r}-N(c-c')^2\right]$$
$$=\frac{1}{Nc}\left[N\Sigma\frac{(x_r-c'a_r)^2}{a_r}-(Nc-Nc')^2\right].$$

As Nc is the total number of cross-overs, the calculation is simple.

TABLE XVII.

Analysis of linkage data from $\frac{\text{PF}}{\text{pf}}$ ♀.

Family	a_r	x_r	$c'a_r$	$x_r-c'a_r$	$\frac{(x_r-c'a_r)^2}{a_r}$
227/31	86	14	12·9	+1·1	0·0148
229/31	216	28	32·4	−4·4	0·0896
231/31	236	30	35·4	−5·4	0·1236
233/31	234	37	35·1	+1·9	0·0151
235/31	369	57	55·35	+1·65	0·0074
420/32	112	16	16·8	−0·8	0·0057
·	1253	182			0·2562

Table XVII gives the requisite calculations for the families derived from $\frac{\text{PF}}{\text{p f}}$ ♀ × $\frac{\text{pf}}{\text{pf}}$ ♂. Here $c=\frac{182}{1253}=0{\cdot}1453$, so we take $c'=0{\cdot}15$.

$$\Sigma\frac{(x_r-c'a_r)^2}{a_r}=0{\cdot}2562,$$

so
$$\chi^2=\frac{1253\times0{\cdot}2562-(182-187{\cdot}95)^2}{182}=1{\cdot}569.$$

This value is low, corresponding to $P=0{\cdot}90$, *i.e.* only once in ten trials should we get as good a fit as the result of random sampling. The number of degrees of freedom is $n=5$.

If more accurate values of P are required, or if it is desired to compare the results of a number of different series of families, we use Wilson and Hilferty's (1931) theorem, which may be stated as follows:

If $$\xi=\left[\left(\frac{\chi^2}{n}\right)^{\frac{1}{3}}+\frac{2}{9n}-1\right]\left(\frac{9n}{2}\right)^{\frac{1}{2}},$$

then ξ is very nearly normally distributed with mean zero and standard error unity. Thus a given value of ξ has the same significance as a departure of $\sigma\xi$ from the expected value in a case where there are only two classes. Here $\xi=-1{\cdot}3095$. That is to say, the probability that ξ should differ from 0 by more than this value is 0·1904. Hence the probability of a greater negative value is 0·0952, and $P=0{\cdot}9048$. Actually this value is not significant. In only one of the 13 sets of families tabulated in Table I is there so large a negative value of ξ.

APPENDIX II.

Methods for the combination of linkage data.

In a favourable case data as to the linkages between two factors are available from six different types of families, namely:

Back-crosses of AB.ab ♀ × ab .ab ♂
,, ,, Ab .aB ♀ × ab .ab ♂
,, ,, ab .ab ♀ × AB.ab ♂
,, ,, ab .ab ♀ × Ab .aB ♂
F_2 ,, AB.ab selfed or *inter se*
,, ,, Ab .aB ,,

Still other data (*e.g.* from AB.ab × Ab.ab) are sometimes available, but these are not present in appreciable numbers in the present case.

The problem is how best to combine the various figures so as to calculate the values of the two unknowns, the cross-over values on the female side and on the male side. The first two data enable us to calculate the female cross-over probability p (one-hundredth of the percentile cross-over value). The second two give us a value for the male cross-over probability q. The coupling F_2 figures gives us an estimate of $(1-p)(1-q)$, the repulsion F_2 of pq.

In each case we can calculate the standard error σ, or more conveniently the quantity $I=\sigma^{-2}$ called by Fisher the amount of information.

In the case of back-crosses, where n is the total number, z the number of cross-overs,

$$I=\frac{n^3}{z\,(n-z)}.$$

In the case of F_2 we find the most likely value of $\theta=pq$ or $(1-p)\,(1-q)$, and then

$$I=\frac{n\,(1+2\theta)}{2\theta\,(1-\theta)\,(2+\theta)}$$

(Fisher and Balmukand, 1928; Haldane, 1919 *a*). We have now the following figures:

Statistic	Estimate	Amount of information
p	p_0	I_p
q	q_0	I_q
pq	r	I_r
$(1-p)\,(1-q)$	c	I_c

Now supposing r exceeds p_0q_0 the most likely values of p and q will exceed p_0 and q_0, and so on. The logarithm of the likelihood (Fisher's definition) of any pair of values of p and q is

$$L=\text{constant}-\tfrac{1}{2}I_p\,(p-p_0)^2-\tfrac{1}{2}I_q\,(q-q_0)^2-\tfrac{1}{2}I_r\,(pq-r)^2 \\ -\tfrac{1}{2}I_c\,[(1-p)\,(1-q)-c]^2.$$

To obtain the likeliest values of p and q we make this a maximum, by putting

$$-\frac{\partial L}{\partial p}=I_p\,(p-p_0)+I_rq\,(pq-r)-I_c\,(1-q)\,[(1-p)\,(1-q)-c]=0,$$

$$-\frac{\partial L}{\partial q}=I_q\,(q-q_0)+I_rp\,(pq-r)-I_c\,(1-p)\,[(1-p)\,(1-q)-c]=0.$$

Now put

$$p-p_0=x,\quad q-q_0=y,\quad c-(1-p_0)\,(1-q_0)=u,\quad r-p_0q_0=v.$$

Then

$$I_px+I_r\,(q_0+y)\,(q_0x+p_0y+xy-v)-I_c\,(1-q_0-y) \\ [-(1-q_0)\,x-(1-p_0)\,y+xy-u]=0,$$

and another similar equation. In most cases it will be found that x, y, u and r are small compared with p_0 and q_0. Hence their squares and products may be neglected, and we have:

$$[I_p+(1-q_0)^2\,I_c+q_0^2I_r]\,x+[(1-p_0)\,(1-q_0)\,I_c+p_0q_0I_r]\,y \\ +(1-q_0)\,I_cu-q_0I_rv=0,$$

$$[(1-p_0)\,(1-q_0)\,I_c+p_0q_0I_r]\,x+[I_q+(1-p_0)^2\,I_c+p_0^2I_r]\,y \\ +(1-p_0)\,I_cu-p_0I_rv=0,$$

whence x, y, p, q may readily be found. In many cases p_0-q_0 is small.

Here if $p_0-q_0=2s$, we have

$$p=p_0-\frac{I_q[(1-s)I_c u-sI_r v]}{I_pI_q+(I_p+I_q)[(1-s)^2I_c+s^2I_r]},$$

$$q=q_0-\frac{I_p[(1-s)I_c u-sI_r v]}{I_pI_q+(I_p+I_q)[(1-s)^2I_c+s^2I_r]}.$$

Both these sets of approximate formulae give results which only differ from the optimal values by a small fraction of their standard errors, but the second set should only be used when p_0-q_0 is small compared with its standard error, as in the case of B and L. If complete accuracy is desired we can use the equations:

$$p-p_0=\frac{I_c}{I_p}[(1-p)(1-q)-c](1-q)-\frac{I_r}{I_p}(pq-r)q,$$

and

$$q-q_0=\frac{I_c}{I_q}[(1-p)(1-q)-c](1-p)-\frac{I_r}{I_q}(pq-r)p.$$

We substitute the values of p_0, q_0, or some other estimates of p, q, in the right-hand side of these equations, and thus obtain approximate values p_1, q_1. We then substitute p_1, q_1 on the right-hand side and obtain p_2, q_2, continuing the process until successive approximations agree. This iteration is rather tedious, but it is a much easier method than the direct solution, which leads to quintic equations for p and q. It has the further advantage that small mistakes in arithmetic are automatically eliminated. However, the approximations first given have been used throughout this paper.

The standard errors of p and q are given by

$$\sigma_p^{-2}=I_p'=-\frac{\partial^2 L}{\partial p^2}=I_p+(1-q)^2I_c+q^2I_r$$

$$\sigma_q^{-2}=I_q'=-\frac{\partial^2 L}{\partial q^2}=I_q+(1-p)^2I_c+p^2I_r.$$

the optimal values of p and q being substituted.

Another set of data assume importance when one factor, say b, is lethal. In this case we can only distinguish between p and q by means of the matings AaBb × aaBb and aaBb × AaBb. Here we also have the results of AaBb *inter se*. But if bb is lethal at such an early stage that **A**bb and aabb cannot be distinguished, families of all these kinds only give estimates based on the ratio of **AB** to **A**bb. Now this is affected by the single-factor ratio **A** : aa, which, as we have shown, is in *Primula sinensis* generally more variable than a linkage value. Hence results on linkage of lethals are likely to be rather unreliable.

In such a case we should have estimates of p and q from AaBb × aaBb and aaBb × AaBb, and of $(1-p)(1-q)$ and pq from AaBb × AaBb. These data could be treated by the methods already given. A few data on p and q derived from AaBb × aaBb and the reciprocal cross exist for non-lethal genes. We have not used them, as they would affect neither the found cross-over values nor their standard errors appreciably. The viability of all the genotypes considered is so good that there appears to be no need to apply the method of balanced inviability so useful in the case of *Drosophila*.

Appendix III.

The relation between map distance and crossing-over.

As a result of double crossing-over the map distance between two factors is always greater than their observed recombination value. But the correction is negligible for short distances. In *Drosophila* it is thus possible, by using a number of factors, to determine the map distance corresponding to say 30 per cent. crossing-over. Where this is not possible we must determine the appropriate correction on the basis of the facts known about double crossing-over. Unfortunately it has been shown by Anderson and Rhoades (1931) and others, that the amount of double crossing-over within a given distance is not constant, being larger near the spindle fibre in *Drosophila*. The theory which follows is admittedly inexact, as it assumes that interference is constant throughout the chromosome.

Let $100x$ be the map distance between two factors.

Let $100y$ be the percentage recombination between two factors.

Let z be the marginal coincidence corresponding to this map distance. That is to say if A, B, C are in that order, and y be the recombination value for A and B, while that for B and C is the small value h, let zyh be the probability of a double cross-over, both between A and B and between B and C.

Then the following conditions must be fulfilled:

(1) $y=x$ approximately when both are small, and $y=\frac{1}{2}$ when x is infinite.

(2) The values of y lie between

$$y=\tfrac{1}{2}(1-e^{-2x}) \quad \text{or} \quad x=\tfrac{1}{2}\log_e(1-2y)$$

corresponding to no interference (Haldane, 1919*b*) and $y=x$ (corresponding to complete interference). Also y is always less than $\frac{1}{2}$.

(3) z is about proportional to y when both are small, and tends to

the value 1 when y tends to the value $\frac{1}{2}$. Anderson and Rhoades found values of z/y for small values of y ranging from about 1·5 to 0·3 in different parts of the X-chromosome. If $y=f(x)$, then the frequency of double cross-overs:

$$zyh=\tfrac{1}{2}\,[f(x)+f(h)-f(x+h)],$$

or in the limit

$$\begin{aligned} z &= \operatorname*{Lt}_{h\to 0}\frac{f(x)+f(h)-f(x+h)}{2hf(x)} \\ &= \frac{1}{2y}\operatorname*{Lt}_{h\to 0}\left[\frac{h+f(x)-f(x+h)}{h}\right] \\ &= \frac{1}{2y}\left(1-\frac{dy}{dx}\right). \end{aligned}$$

Now if we assume that z is everywhere the same function of y, we may take $z=\dfrac{2cy}{1+2\,(c-1)\,y}$, in which case $z/y=2c$ when both are small, and $z=1$ when $y=\frac{1}{2}$. Since $\dfrac{dy}{dx}=1-2yz$,

$$x=\int\frac{dy}{1-2yz}=\int\frac{1+2\,(c-1)\,y}{1+2\,(c-1)\,y-4cy^2}\,dy$$

$$=\frac{\dfrac{1}{c}\log_e(1+2cy)-c\log(1-2y)}{2\,(c+1)} \qquad \ldots\ldots(1).$$

Whereas in the X-chromosome of *Drosophila* c ranges from about 0·15 to 0·7, $c=2$ is an appropriate value for *Primula sinensis*, giving the formula

$$x=\tfrac{1}{12}\log_e(1+4y)-\tfrac{1}{3}\log_e(1-2y) \qquad \ldots\ldots(2).$$

When x is small $x=y+\dfrac{4c}{3}\,y^3$ approximately, so the curve $y=f(x)$ has a point of inflexion at 0, touching $y=x$. On the other hand the curve given by Haldane (1919 *b*) has single contact with $y=x$, and leads to a finite value of z when x vanishes. Using equation (2) we find the double cross-overs calculated in Table IX. The sum of the double cross-overs from the SBG plants calculated from this formula is 329, agreeing well with the observed value of 323. Thus the equation is at least a rough guide to the facts. Corresponding values of x and y were tabulated. Table XVIII is extracted from this table. Using it the map distances of Table XIX were calculated, and Fig. 1 is constructed on this basis. It is to be noted that formulae of the type here developed do not enable

the prediction of triple crossing-over, or of interference between two separated segments of a chromosome, such as SB and GL.

TABLE XVIII.

Calculated relation between map distance and percentage recombination.

Recombination:											
100y	0	5	10	15	20	25	30	35	40	45	50
Map distance:											
100x	0	5·03	10·24	15·81	21·03	28·88	37·11	47·43	61·56	85·34	∞

TABLE XIX.

Calculated map distances.

Factors	Recombination value	Map distance	Factors	Recombination value	Map distance
SB ♀	6·25	6·33	SB ♂	11·55	11·92
SG ♀	34·40	46·06	SG ♂	41·03	65·37
SL ♀	37·53	54·03	SL ♂	41·45	67·07
BG ♀	32·14	41·21	BG ♂	35·01	47·45
BL ♀	36·73	51·77	BL ♂	36·86	52·11
GL ♀	3·61	3·61	GL ♂	1·82	1·82

TABLE XX.

Provisional loci.

	♀	♂		♀	♂		♀	♂		♀	♂
S	0	0	P	0	0	M	0	0	O	0	0
B	6·3	11·9	W	8	14	Y	28	17·6	Mp	27	24
X	21	26	F	16	25	H	34	—			
G	47	68	Ch	27	35	K	37	—			
L	50·6	69·8									

Summary.

1. Fifteen factors have been located on four out of the twelve chromosomes. Some other linkages are suspected, and a new case of multiple allelomorphism or very close linkage is described.

2. As compared with *Drosophila*, interference is definitely less marked, though it certainly occurs.

3. In two regions linkage is tighter on the male than on the female side, in two looser, while in two others it is nearly the same. Apart from this the figures are inadequate for a judgment.

4. In one chromosome the genetical data from the tetraploid permit the rough location of a spindle attachment.

5. The linkage values found in different families show a remarkable constancy.

6. New mathematical methods are given for dealing with goodness of fit, combination of linkage data, and allowance for double cross-overs when mapping.

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