LINKAGE IN THE TETRAPLOID *PRIMULA SINENSIS*.

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CONTENTS.

Introduction.

Linkage has so far been almost exclusively studied in diploids and allopolyploids such as wheat. In the latter the phenomenon is not essentially different from that in diploids, as each chromosome, save for rare exceptions, has a definite mate. The only case of true polyploid linkage so far studied is that in the triplo-**X** *Drosophila melanogaster* of Bridges and Anderson (1925). No linked factors were known in autotetraploid plants other than *Primula sinensis* until 1930, but our colleague Dr Sansome is now studying linkage in tetraploid tomatoes.

The present work was begun in 1909 by the late R. P. Gregory. Sverdrup Sömme (1930) has analysed the data up to 1927. We incorporate counts of 2867 more plants, but reject some of her data on various grounds.

The factors here considered are **S**, **B** and **G**. **S** converts a pin plant, with long style and short stamens, into a thrum with short style and long stamens. **B** converts red flower pigment into magenta, **G** inhibits the formation of anthocyanin pigment in the centre of the flower, producing a green stigma and ovary in place of a red. In the diploid they are completely dominant. In the tetraploid **S** is completely so, but **Bbbb** and **Gggg**, though generally easily distinguished from **bbbb** and **gggg**, are on the whole not so different from them as are **BBBB** and **GGGG**. There is no possibility of a mistake in scoring **S**. In some families **Bbbb** and **bbbb** may have been confused. It is possible, though not very likely, that errors may have been made regarding **G**. No attempt has been made to separate different grades of dominant, *e.g.* **BBbb** and **Bbbb**, though in some cases this was partly possible.

The linkage values of these factors in the diploid are based on a mass of material, partly given by Gregory, de Winton and Bateson (1923). Table I is based entirely on back-cross data as regards the diploid; the F_2 data are concordant, but do not enable a distinction to be made between the male and female sides of the plant. The data on which the tetraploid figures are based will be given later.

TABLE I.

Cross-over values per cent., with standard errors.

Factors	Diploid ♀	Diploid ♂	Tetraploid ♀	Tetraploid ♂
SB	7·35 ±0·40	12·91 ±0·50	8·01 ±0·69	8·41 ±0·99
SG	33·29 ±0·74	40·47 ±0·78	37·58 ±1·92	38·91 ±2·23
BG	31·15 ±0·53	36·24 ±0·68	35·18 ±1·85	34·38 ±2·17

In order to understand the linkage data we must first consider the genetical behaviour of the factors one at a time. Apart from aneuploids, *e.g.* plants with $4n + 1$ (49) chromosomes, the following types of zygote are to be expected with regard to a single pair of allelomorphs **X** and **x**: **XXXX**, quadruplex; **XXXx**, triplex; **XXxx**, duplex; **Xxxx**, simplex; **xxxx**, nulliplex.

Quadruplex and triplex plants give no recessive offspring, duplex by nulliplex give 5 dominant : 1 recessive, simplex by nulliplex 1 dominant : 1 recessive. In Table II actual figures are given for the factors concerned. These figures do not represent all the material available, but only those in which the composition of the dominant parent was known from its ancestry. All the duplex plants included in them were from the cross **XXXX** × **xxxx** or reciprocal. The only element of doubt here is the possibility of an alleged **XXXX** grand-parent having been **XXXx**, such triplex plants being indistinguishable from quadruplex by a single generation of breeding, and being eliminated rather slowly on self-fertilisation. Plants known by their genetic behaviour, but not their ancestry, to have been **XXxx** are excluded. Such plants occurred, for example, among the progeny when **XXxx** was selfed.

Similarly the only **Xxxx** plants whose progeny is included are those from **Xxxx** × **xxxx** or the reciprocal cross, the constitution of the simplex grand-parent being assumed, if necessary, from its genetical behaviour. Dominant progeny of 73 crosses of simplex × nulliplex have been tested, and all have proved simplex. On the hypothesis of random assortment of chromatids (Haldane, 1930) one in thirteen would have been duplex.

TABLE II.

Single factor ratios.

Parents	No. of families	Dominant	Recessive	D	$D \div \sigma$
$\mathbf{Ss}_3 \times \mathbf{Ss}_3$	40	1282	395	−24·25	1·20
$\mathbf{Bb}_3 \times \mathbf{Bb}_3$	17	358	124	+ 3·5	0·37
$\mathbf{Gg}_3 \times \mathbf{Gg}_3$	10	265	91	+ 2	0·24
$\mathbf{Ss}_3 \times \mathbf{s}_4$	75	1001	1009	+ 4	0·18
$\mathbf{Bb}_3 \times \mathbf{b}_4$	38	559	542	− 8·5	0·51
$\mathbf{Gg}_3 \times \mathbf{g}_4$	25	368	371	+ 1·5	0·11
$\mathbf{s}_4 \times \mathbf{Ss}_3$	45	456	534	+39	2·48
$\mathbf{b}_4 \times \mathbf{Bb}_3$	28	291	380	+44·5	3·43
$\mathbf{g}_4 \times \mathbf{Gg}_3$	24	293	315	+11	0·89
$\mathbf{S}_2\mathbf{s}_2 \times \mathbf{S}_2\mathbf{s}_2$	1	44	2	+ 0·7	0·65
$\mathbf{G}_2\mathbf{g}_2 \times \mathbf{G}_2\mathbf{g}_2$	23	1204	44	+10	1·74
$\mathbf{G}_2\mathbf{g}_2 \times \mathbf{g}_4$	34	643	125	− 3	0·29
$\mathbf{g}_4 \times \mathbf{G}_2\mathbf{g}_2$	9	87	22	+ 3·8	0·98

Table II shows the genetical behaviour of simplex and duplex plants. The results are not independent, owing to linkage. It will be seen that the only really serious deviations from expectation occur in the cross of $\mathbf{x}_4 \times \mathbf{Xx}_3$, the heterozygotes giving an excess of recessive gametes on the male side. The fact that the same families are included in the **B** and **S** totals accounts for the similar discrepancy in both cases, since **B** and **S** were coupled in many of the plants. Five $\mathbf{Bb}_3$ plants as males gave 66 **B**, 106 **b**, which accounts for nearly half the discrepancy. Used as females they gave 90 **B**, 85 **b**. We have clearly to deal with a case of anisogeny, the **B** pollen grains being handicapped while the **B** ovules are not. A possible explanation is that these plants were **Bbbbb**, *i.e.* 49 chromosome plants, and that as in *Datura* $2n + 1$ ovules are functional, $2n + 1$ pollen not so in competition with $2n$ pollen. In this case the ovules would give a ratio of 1 **B** : 1 **b**, the pollen grains 2 **B** : 3 **b**, which agrees with observation. If the divergence from expectation were due to random pairing of chromatids we should expect similar gametic ratios on both sides, for the equality of linkage values suggests that meiosis is similar on the two sides of the plant. If non-disjunction of the **SBG** chromosome is at all common we should expect to find **Bbbbb** plants among the parents of Table II; on the other hand **Bbbb** × **bbbb** or the reciprocal cross could not give **BBbbb** apart from double reduction.

Exceptions due to double reduction may occur, but they are too rare to be considered in an admittedly preliminary theory of linkage.

Hence in what follows we assume that two chromatids from the same chromosome always go into different gametes. This is borne out by the behaviour of linked factors. The further question whether two chromatids which have paired and exchanged factors by crossing-over may enter the same gamete is considered later. Both these anomalies would involve non-disjunction.

THEORY OF LINKAGE IN TETRAPLOIDS.

In a diploid organism heterozygous for two factors we can only study two types of gametic series, those characteristic of coupling and repulsion, which are closely related, and their relation could be deduced on many different theories of segregation. For example, it was consistent with the reduplication theory, or with several different theories as to the relation between factors and chromosomes.

In the tetraploid, however, there should be seven distinct types of gametic series: (1) single coupling, (2) single repulsion, (3) asymmetrical coupling, (4) asymmetrical repulsion, (5) double coupling, (6) double repulsion, (7) coupling *and* repulsion. It will be difficult to construct zygotes giving combined coupling and repulsion, or to identify them with certainty if they have been constructed.

We consider only the ratios to be expected in the case of completely dominant factors. In order to obtain visible segregation the number of these factors in a zygote must be one or two. Suppose two factors **X** and **Y** to be linked, crossing-over occurring in the formation of a proportion p of the gametes; and a plant known from its genetical performance to be of the composition

$$\begin{matrix}\mathbf{XY}\\ \mathbf{xy}\\ \mathbf{xy}\\ \mathbf{xy}\end{matrix}$$

Then if, after crossing-over has occurred, two chromosomes can enter the same gamete, some of its **XY** offspring when it is crossed with $(\mathbf{xy})_4$ will exhibit repulsion of **X** and **Y**. As will be seen later, such an event is rare, if it occurs at all. In what follows we will assume that it does not occur, *i.e.* that after two chromosomes have paired, they must proceed to different poles. The absence of such a conversion of coupling into repulsion also renders pairing of three chromosomes leading to "progressive" crossing-over unlikely, and further reasons are given later to show that it is a rare or non-existent phenomenon.

Case 1. Single coupling.

$$\begin{matrix}\mathbf{XY}\\\mathbf{xy}\\\mathbf{xy}\\\mathbf{xy}\end{matrix}$$

The chromosome containing **X** and **Y** must pair with one of the others. Crossing-over occurs in p of the total cases, the gametic series is therefore:

$$(1-p)\begin{matrix}\mathbf{XY}\\\mathbf{xy}\end{matrix} : p\begin{matrix}\mathbf{Xy}\\\mathbf{xy}\end{matrix} : p\begin{matrix}\mathbf{xY}\\\mathbf{xy}\end{matrix} : (1-p)\begin{matrix}\mathbf{xy}\\\mathbf{xy}\end{matrix}$$

a series similar to that of the diploid.

Case 2. Single repulsion.

$$\begin{matrix}\mathbf{Xy}\\\mathbf{xY}\\\mathbf{xy}\\\mathbf{xy}\end{matrix}$$

Calling the four chromosomes A, B, C and D, in two-thirds of all cases A does not pair with B, so the gametes are:

$$1\begin{matrix}\mathbf{Xy}\\\mathbf{xY}\end{matrix} : 1\begin{matrix}\mathbf{Xy}\\\mathbf{xy}\end{matrix} : 1\begin{matrix}\mathbf{xY}\\\mathbf{xy}\end{matrix} : 1\begin{matrix}\mathbf{xy}\\\mathbf{xy}\end{matrix}$$

In the remaining one-third A and B pair, so there is a chance of crossing-over, the gametes being:

$$p\begin{matrix}\mathbf{XY}\\\mathbf{xy}\end{matrix} : (1-p)\begin{matrix}\mathbf{Xy}\\\mathbf{xy}\end{matrix} : (1-p)\begin{matrix}\mathbf{xY}\\\mathbf{xy}\end{matrix} : p\begin{matrix}\mathbf{xy}\\\mathbf{xy}\end{matrix}$$

Hence the total gametic series is:

$$1\begin{matrix}\mathbf{Xy}\\\mathbf{xY}\end{matrix} : p\begin{matrix}\mathbf{XY}\\\mathbf{xy}\end{matrix} : (2-p)\begin{matrix}\mathbf{Xy}\\\mathbf{xy}\end{matrix} : (2-p)\begin{matrix}\mathbf{xY}\\\mathbf{xy}\end{matrix} : (1+p)\begin{matrix}\mathbf{xy}\\\mathbf{xy}\end{matrix}$$

Case 3. Asymmetrical coupling.

$$\begin{matrix}\mathbf{XY}\\\mathbf{Xy}\\\mathbf{xy}\\\mathbf{xy}\end{matrix}$$

Here in one-third of all cases AB, CD pair and the gametes are:

$$1\begin{matrix}\mathbf{XY}\\\mathbf{xy}\end{matrix} : 1\begin{matrix}\mathbf{Xy}\\\mathbf{xy}\end{matrix}$$

In the other cases crossing-over may occur between A and C or D, and the resulting chromosome has an equal chance of entering an **Xy** or an **xy** gamete. The total gametic series is therefore:

$$(1-p)\begin{matrix}\mathbf{XY}\\\mathbf{Xy}\end{matrix} : (2-p)\begin{matrix}\mathbf{XY}\\\mathbf{xy}\end{matrix} : p\begin{matrix}\mathbf{Xy}\\\mathbf{xY}\end{matrix} : p\begin{matrix}\mathbf{Xy}\\\mathbf{Xy}\end{matrix} : 2\begin{matrix}\mathbf{Xy}\\\mathbf{xy}\end{matrix} : p\begin{matrix}\mathbf{xY}\\\mathbf{xy}\end{matrix} : (1-p)\begin{matrix}\mathbf{xy}\\\mathbf{xy}\end{matrix}$$

Case 4. Asymmetrical repulsion.

$$\begin{matrix}\mathbf{Xy}\\\mathbf{Xy}\\\mathbf{xY}\\\mathbf{xy}\end{matrix}$$

In one-third of all cases *AB*, *CD* pair and the gametes are:

$$1\begin{matrix}\mathbf{Xy}\\\mathbf{xY}\end{matrix} : 1\begin{matrix}\mathbf{Xy}\\\mathbf{xy}\end{matrix}$$

In the other two-thirds pairing of *C* with *A* or *B* renders crossing-over possible, the resulting chromosomes as above entering a gamete with **Xy** or **xy** in equal numbers. The total gametic series is thus:

$$p\begin{matrix}\mathbf{XY}\\\mathbf{Xy}\end{matrix} : p\begin{matrix}\mathbf{XY}\\\mathbf{xy}\end{matrix} : (2-p)\begin{matrix}\mathbf{Xy}\\\mathbf{xY}\end{matrix} : (1-p)\begin{matrix}\mathbf{Xy}\\\mathbf{Xy}\end{matrix} : 2\begin{matrix}\mathbf{Xy}\\\mathbf{xy}\end{matrix} : (1-p)\begin{matrix}\mathbf{xY}\\\mathbf{xy}\end{matrix} : p\begin{matrix}\mathbf{xy}\\\mathbf{xy}\end{matrix}$$

Case 5. Double coupling.

$$\begin{matrix}\mathbf{XY}\\\mathbf{XY}\\\mathbf{xy}\\\mathbf{xy}\end{matrix}$$

In one-third of all cases *AB*, *CD* pair and the gametes are $\begin{matrix}\mathbf{XY}\\\mathbf{xy}\end{matrix}$. In two-thirds of the cases crossing-over may occur. The following question now arises. Does the fact that crossing-over has occurred between chromosomes *A* and *C* alter the probability of crossing-over between *B* and *D*? This probability would be increased if certain variable conditions in the nucleus as a whole favoured crossing-over in both cases. It would be diminished if, for example, only a finite amount of energy was available for twisting or breaking the chromosomes, and this might be concentrated on one pair or the other. In what follows we shall assume that the probabilities are independent, an hypothesis which agrees fairly well with experience. The gametic output where crossing-over is possible is therefore symbolised by

$$[(1-p)\,\mathbf{XY} : p\,\mathbf{Xy} : p\,\mathbf{xY} : (1-p)\,\mathbf{xy}]^2$$

and the total gametic series is:

$$(1-2p+p^2)\begin{matrix}\mathbf{XY}\\\mathbf{XY}\end{matrix} : (2p-2p^2)\begin{matrix}\mathbf{XY}\\\mathbf{Xy}\end{matrix} : (2p-2p^2)\begin{matrix}\mathbf{XY}\\\mathbf{xY}\end{matrix} :$$

$$2\,(2-2p+p^2)\begin{matrix}\mathbf{XY}\\\mathbf{xy}\end{matrix} : 2p^2\begin{matrix}\mathbf{Xy}\\\mathbf{xY}\end{matrix} : p^2\begin{matrix}\mathbf{Xy}\\\mathbf{Xy}\end{matrix} : (2p-2p^2)\begin{matrix}\mathbf{Xy}\\\mathbf{xy}\end{matrix} :$$

$$p^2\begin{matrix}\mathbf{xY}\\\mathbf{xY}\end{matrix} : (2p-p^2)\begin{matrix}\mathbf{xY}\\\mathbf{xy}\end{matrix} : (1-p)^2\begin{matrix}\mathbf{xy}\\\mathbf{xy}\end{matrix}$$

In the event of a positive or negative correlation between the two cross-overs the terms whose coefficients are divisible by p would be increased or diminished respectively.

Case 6. Double repulsion.

$$\begin{matrix}\mathbf{Xy}\\\mathbf{Xy}\\\mathbf{xY}\\\mathbf{xY}\end{matrix}$$

In one-third of all cases *AB, CD* pair and the gametes are all $\begin{matrix}\mathbf{Xy}\\\mathbf{xY}\end{matrix}$. In the remaining two-thirds crossing-over may occur. If it is independent the output is symbolised by

$$[p\ \mathbf{XY} : (1-p)\ \mathbf{Xy} : (1-p)\ \mathbf{xY} : p\ \mathbf{xy}]^2$$

and the total gametic series is:

$$p^2 \begin{matrix}\mathbf{XY}\\\mathbf{XY}\end{matrix} : (2p-2p^2) \begin{matrix}\mathbf{XY}\\\mathbf{Xy}\end{matrix} : (2p-2p^2) \begin{matrix}\mathbf{XY}\\\mathbf{xY}\end{matrix} : 2p^2 \begin{matrix}\mathbf{XY}\\\mathbf{xy}\end{matrix} : (4-4p+2p^2) \begin{matrix}\mathbf{Xy}\\\mathbf{xY}\end{matrix} :$$

$$(1-p)^2 \begin{matrix}\mathbf{Xy}\\\mathbf{Xy}\end{matrix} : (2p-2p^2) \begin{matrix}\mathbf{Xy}\\\mathbf{xy}\end{matrix} : (1-p)^2 \begin{matrix}\mathbf{xY}\\\mathbf{xY}\end{matrix} : (2p-2p^2) \begin{matrix}\mathbf{xY}\\\mathbf{xy}\end{matrix} : p^2 \begin{matrix}\mathbf{xy}\\\mathbf{xy}\end{matrix}$$

subject to the above reservation.

Case 7. Coupling and repulsion.

$$\begin{matrix}\mathbf{XY}\\\mathbf{Xy}\\\mathbf{xY}\\\mathbf{xy}\end{matrix}$$

In one-third of all cases *AB, CD* pair and the gametes are:

$$1 \begin{matrix}\mathbf{XY}\\\mathbf{xY}\end{matrix} : 1 \begin{matrix}\mathbf{XY}\\\mathbf{xy}\end{matrix} : 1 \begin{matrix}\mathbf{Xy}\\\mathbf{xY}\end{matrix} : 1 \begin{matrix}\mathbf{Xy}\\\mathbf{xy}\end{matrix}$$

In one-third of all cases *AC, BD* pair and the gametes are:

$$1 \begin{matrix}\mathbf{XY}\\\mathbf{Xy}\end{matrix} : 1 \begin{matrix}\mathbf{XY}\\\mathbf{xy}\end{matrix} : 1 \begin{matrix}\mathbf{Xy}\\\mathbf{xY}\end{matrix} : 1 \begin{matrix}\mathbf{xY}\\\mathbf{xy}\end{matrix}$$

In one-third of all cases *AD, CB* pair and in the absence of correlation the gametic series is represented by

$$[p\ \mathbf{XY} : (1-p)\ \mathbf{Xy} : (1-p)\ \mathbf{xY} : p\ \mathbf{xy}]$$
$$\times [(1-p)\ \mathbf{XY} : p\ \mathbf{Xy} : p\ \mathbf{xY} : (1-p)\ \mathbf{xy}]$$

Hence the total gametic series is:

$$(p-p^2)\genfrac{}{}{0pt}{}{\mathbf{XY}}{\mathbf{XY}} : (2-2p+2p^2)\genfrac{}{}{0pt}{}{\mathbf{XY}}{\mathbf{Xy}} : (2-2p+2p^2)\genfrac{}{}{0pt}{}{\mathbf{XY}}{\mathbf{xY}} :$$

$$(2+2p-2p^2)\genfrac{}{}{0pt}{}{\mathbf{XY}}{\mathbf{xy}} : (2+2p-2p^2)\genfrac{}{}{0pt}{}{\mathbf{Xy}}{\mathbf{xY}} : (p-p^2)\genfrac{}{}{0pt}{}{\mathbf{Xy}}{\mathbf{Xy}} :$$

$$(2-2p+2p^2)\genfrac{}{}{0pt}{}{\mathbf{Xy}}{\mathbf{xy}} : (p-p^2)\genfrac{}{}{0pt}{}{\mathbf{xY}}{\mathbf{xY}} : (2-2p+2p^2)\genfrac{}{}{0pt}{}{\mathbf{xY}}{\mathbf{xy}} : (p-p^2)\genfrac{}{}{0pt}{}{\mathbf{xy}}{\mathbf{xy}}$$

provided that, in the third case, crossing-over is not correlated.

Since in the case of complete dominance, the various classes of gamete containing at least one **X** and **Y** produce indistinguishable zygotes, the above results may be summarised in Table III. p and q are the cross-over ratios on the two sexual sides of the plant.

TABLE III.

Type of zygote	Types of gametes	Gametes in general	Gametes, $p=0$	Gametes, $p=\frac{1}{2}$	Gametes in absence of linkage	Zygotic ratio on selfing
1. $\mathbf{XY}.(\mathbf{xy})_3$	**XY**	$1-p$	1	1	1	$2+(1-p)(1-q)$
	Xy	p	0	1	1	$1-(1-p)(1-q)$
	xY	p	0	1	1	$1-(1-p)(1-q)$
	xy	$1-p$	1	1	1	$(1-p)(1-q)$
2. $\mathbf{Xy}.\mathbf{xY}.(\mathbf{xy})_3$	**XY**	$1+p$	1	1	1	$18+(1+p)(1+q)$
	Xy	$2-p$	2	1	1	$9-(1+p)(1+q)$
	xY	$2-p$	2	1	1	$9-(1+p)(1+q)$
	xy	$1+p$	1	1	1	$(1+p)(1+q)$
3. $\mathbf{XY}.\mathbf{Xy}.(\mathbf{xy})_2$	**XY**	$3-p$	3	5	5	$26+(1-p)(1-q)$
	Xy	$2+p$	2	5	5	$9-(1-p)(1-q)$
	xY	p	0	1	1	$1-(1-p)(1-q)$
	xy	$1-p$	1	1	1	$(1-p)(1-q)$
4. $(\mathbf{Xy})_2.\mathbf{xY}.\mathbf{xy}$	**XY**	$2+p$	2	5	5	$26+pq$
	Xy	$3-p$	3	5	5	$9-pq$
	xY	$1-p$	1	1	1	$1-pq$
	xy	p	0	1	1	pq
5. $(\mathbf{XY})_2.(\mathbf{xy})_2$	**XY**	$5-2p+p^2$	5	17	25	$34+(1-p)^2(1-q)^2$
	Xy	$2p-p^2$	0	3	5	$1-(1-p)^2(1-q)^2$
	xY	$2p-p^2$	0	3	5	$1-(1-p)^2(1-q)^2$
	xy	$1-2p+p^2$	1	1	1	$(1-p)^2(1-q)^2$
6. $(\mathbf{Xy})_2.(\mathbf{xY})_2$	**XY**	$4+p^2$	4	17	25	$34+p^2q^2$
	Xy	$1-p^2$	1	3	5	$1-p^2q^2$
	xY	$1-p^2$	1	3	5	$1-p^2q^2$
	xy	p^2	0	1	1	p^2q^2
7. $\mathbf{XY}.\mathbf{Xy}.\mathbf{xY}.\mathbf{xy}$	**XY**	$8+p-p^2$	4	33	25	$136+pq(1-p)(1-q)$
	Xy	$2-p+p^2$	1	7	5	$4-pq(1-p)(1-q)$
	xY	$2-p+p^2$	1	7	5	$4-pq(1-p)(1-q)$
	xy	$p-p^2$	0	1	1	$pq(1-p)(1-q)$

For purposes of calculation it is convenient to put $1-p=P$, $1-q=Q$.

The gametic series in double coupling may then be written $4+P^2 : 1-P^2 : 1-P^2 : P^2$, and the expressions for the following zygotic series may be simplified:

Single coupling, $2+PQ : 1-PQ : 1-PQ : PQ$.
Asymmetrical coupling, $26+PQ : 9-PQ : 1-PQ : PQ$.
Double coupling, $34+P^2Q^2 : 1-P^2Q^2 : 1-P^2Q^2 : P^2Q^2$.
Coupling and repulsion, $136+pqPQ : 4-pqPQ : 4-pqPQ : pqPQ$.

The second column gives the gametic series in general, the third the expected ratios when linkage is so strong that crossing-over may be neglected, the fourth when linkage is so weak that crossing-over amounts to 50 per cent. In the fifth column the ratios are given which are found when the factors are in different chromosomes. In the last column are given the zygotic ratios to be expected on selfing. In the case of single coupling the ratios are the same as in a diploid. But in the case of single repulsion this is not so, since two factors in different chromosomes can still enter the same gamete. The asymmetrical cases call for no special comment.

Whereas in the first four cases no difference is to be expected when the factors, though in the same chromosome, are far apart, from the ratios obtained when they are in different chromosomes, this is not so in the last three. If the factors are in different chromosomes each tetrad of homologous chromosomes can pair in three ways giving two pairs each, so the total number of distinct cases to be considered is 36. But if the factors are far apart in the same chromosome each of the six possible pairs of chromosomes can produce one, two or four different types of gametes; the total number of cases is therefore 24 or 48. It is thus theoretically possible, in a tetraploid plant, to distinguish between 50 per cent. crossing-over and the absence of linkage.

Similar calculations have been made to meet the possibility that, after pairing, the chromosomes can enter gametes at random, so that in one-third of all cases, two chromosomes which have paired so as to permit of crossing-over may enter the same gamete. The expected ratios are somewhat different. As, however, it will be shown that this event occurs rarely if ever in *Primula sinensis*, the possibility need not be further considered here. It is however possible that it may occur in other tetraploid organisms, or that a state of affairs may be found in them intermediate between the above condition and that here described. If after pairing, chromosomes always went to the same pole, only **XY** and **xy** gametes would be found in the case of single or double coupling. This, of course, is not the case.

When three factors are concerned, matters are much more complicated: 44 possible zygotic types must be considered. Moreover two different types of double crossing-over are theoretically possible. Consider four homologous chromosomes *A*, *B*, *C*, *D*, in a zygote

XYZ
xyz
xyz
xyz

Chromosome *A* may pair with *B*, crossing-over twice, and giving **XyZ** and **xYz** gametes. Or it may pair with both *B* and *C*, giving **Xyz**, **xYz** and **xyZ**. Both these types of crossing-over were found by Bridges and Anderson (1925) in the triploid *Drosophila melanogaster*, the first being termed recurrent, the second progressive. In the case of progressive crossing-over in a tetraploid we should expect that, as the result of a situation such as that shown in Fig. 1, the two gametes formed would be $\begin{matrix}\mathbf{xYz}\\ \mathbf{xyz}\end{matrix}$ and $\begin{matrix}\mathbf{Xyz}\\ \mathbf{xyZ}\end{matrix}$. The fact that in such a case **X** and **Z** would exhibit repulsion in the progeny shows that such cases, if they occur, are rare. If they are at all frequent, however, **XyZ** and **xYz** gametes should be more commonly produced by the zygote

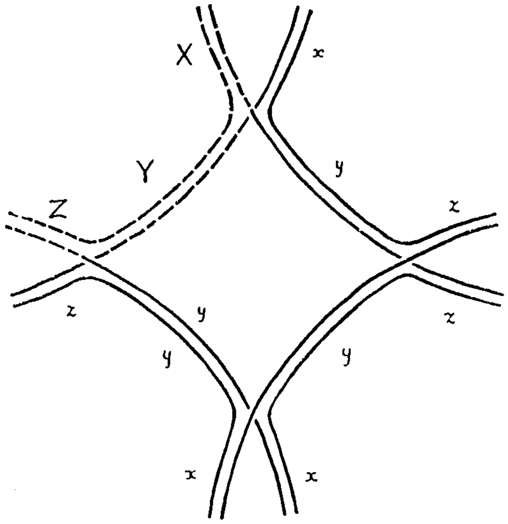

Fig. 1. Configuration in the diplotene stage which might yield $\begin{matrix}\mathbf{Xyz}\\ \mathbf{xyZ}\end{matrix}$ gametes. The chromosome containing the three dominants is dotted.

$$\begin{matrix}\mathbf{XYZ}\\ \mathbf{xyz}\\ \mathbf{xyz}\\ \mathbf{xyz}\end{matrix}$$

than by $\begin{matrix}\mathbf{XYZ}\\ \mathbf{xyz}\end{matrix}$, provided that the cross-over values are the same in both. Double cross-overs are not more common in the tetraploid, not at least to any significant extent. Hence it is considered that, for the present, a theory based on crossing-over involving two chromosomes only, which is not presented as final, will cover all the facts found, to a first approximation.

In view of the somewhat complicated nature of the theory, it is perhaps worth pointing out simpler, if less rigorous, methods of calculating some of the above gametic and zygotic series. Consider the case of double repulsion. The **yy** gametes form one-sixth of the whole, from the single factor theory. But in order that a **yy** gamete should also be **xx**, crossing-over must have occurred twice. The chance of this is p^2: hence the ratio of **Xyy** to **xxyy** gametes is $1 - p^2 : p^2$. The relative proportion of **xxY** gametes is also $1 - p^2$. But since the total proportion of **X** gametes is five for every one **xx**, there must be $4 + p^2$ **XY** gametes. Similarly the proportion of **xxyy** gametes in double coupling is P^2, this being the chance that no crossing-over has occurred on two distinct occasions.

In the corresponding zygotic series the proportions of bottom recessives are $\frac{p^2q^2}{36}$ and $\frac{P^2Q^2}{36}$. In the first case crossing-over must have occurred twice on the male and twice on the female side (a most improbable occurrence), in the second it must have failed to occur on four independent occasions. So that even in a doubly coupled zygote, double recessives rarely occur as a result of selfing. Thus if the cross-over value in each sex is $33\frac{1}{3}$ per cent., $p = \frac{1}{3}$, $P = \frac{2}{3}$, therefore the proportion of double recessives is $\frac{1}{36}\left(\frac{2}{3}\right)^4$, or 4 in 729. This is greater than the proportion of 1 in 1296 expected in the absence of linkage, but is still small.

EXPERIMENTAL RESULTS.

In the interpretation of the experimental results there is a certain danger of circularity in the argument. Thus a number of plants, from their genetical behaviour, agree with the expectation on the assumption that they are of the composition

SBG
sbg
sbg
sbg

Some of these plants are derived from a cross between a triple recessive plant and a triple dominant of uncertain composition. Others arise from selfing, and so on. Only their genetical behaviour makes their composition more or less certain. On the other hand, many such plants are derived from the cross of a plant known from its ancestry, genetical behaviour, or both, to be of the above composition, crossed with a triple recessive. On the theory here developed, all plants in such families, provided that they carry the three dominants, should be of the above composition, unless some exceptional event such as progressive crossing-over has occurred. Such plants, and no others, may legitimately be used to test the theory.

We may however legitimately include with them other plants of similar genetical behaviour, provided their offspring occur in the theoretical proportions, and use the total of such families for the purpose of calculating linkage intensities. In the tables three distinct classes of parents are considered.

(*a*) Parents whose genetic composition can be predicted from those of their ancestors on the assumption that neither double reduction, non-disjunction, nor progressive crossing-over has occurred.

(*b*) Parents in which the ancestry does not suffice to determine the number of factors, but where the linkages are certain once the numbers of factors are known. Now the number of factors (*i.e.* whether the plant is simplex or duplex) can be determined from the ratios in which the single factors segregate in their progeny. In families of ten or more plants there is very little chance of confusing a 1 : 1 with a 5 : 1 ratio, and little chance of confusing a 3 : 1 with a 35 : 1. Hence the progeny of such plants furnish reliable data on linkage. For example $161^1/24$ was derived from a cross between **SSSsBBbb** and **ssssbbbb**, and being a thrum magenta, was either duplex or simplex in **S** and **B**. Crossed with an **ssssbbbb** plant it gave 18 **S**, 2 **s** and 7 **B**, 13 **b**. Hence its composition was **SSssBbbb**. But since one of the gametes which formed it was **ssbb**, its own composition was necessarily

SB
Sb
sb
sb

Most of our data on asymmetrical linkage are derived from such plants, though it would be theoretically possible to make them from a cross between **SSSSBbbb** (derived from a cross of **SSSSBbbb** and **SSSSbbbb**) and **ssssbbbb**. Such a parent would be of class (*a*).

(*c*) Parents whose composition is only deducible from their offspring. As an example

SBG
sbg
sbg
sbg

selfed has given several plants which behaved as

SBG
SBG
sbg
sbg

But even after the single-factor ratios were determined they might as well have been of composition

SBG or of **SBg**
SBg **SBg**
sbG **sbG**
sbg **sbG**

to name only two possibilities.

We are quite aware that the inclusion of class (*b*) leads to a certain distortion of the single factor ratios, and class (*c*) may also distort the linkage ratios, owing to the omission, in each case, of parents of uncertain composition. Nevertheless we think their inclusion justifiable, with the above caution. It will be seen that even if attention is confined to the class (*a*) plants, the general features of the linkage are quite clear. We have allowed ourselves the inclusion of a few families of 1930 which are not quite complete, certain plants not yet having flowered. These are not included in the totals of Table II, but we consider that on the whole more is gained than lost by including them in Tables IV–XI.

Origin of the plants considered.

Tetraploids of the following origins have been used:

(*a*) Gregory's (1914) **GX** race which originated in a plant from Messrs Sutton in 1909. This plant lacked **S** and was at least duplex for **B**, **G**, **D** (white), and **Y** (palmate as opposed to fern leaf).

(*b*) Gregory's (1914) **GT** race which originated in 1911 from a cross made by him between two diploids. It was at least duplex for **S**, **B** and **G**, and also carried **D**.

(*c*) Sutton's "Symmetry," introduced into the experiments in 1920, and Sutton's "Mosscurl" in 1922. Both lacked **S** and **B** and were at least triplex for **G**. Most of our numbers for **S**, **G** asymmetrical repulsion come from these races crossed to others lacking **G** and simplex for **S**.

Linkage between two factors.

The single coupling figures (Table IV) require little comment. Only two of the families considered are in any way abnormal. Among the class (*a*) families from $\mathbf{SB}\,.\,(\mathbf{sb})_3 \times (\mathbf{sb})_4$ occurs one (149/22) consisting of

19 **SB**, 20 **Sb**, 11 **sB**, 19 **sb**. It seems probable that its parent $43^1/21$ was of the composition mentioned, and it behaved more normally in another similar cross, giving 5 **SB**, 1 **Sb**, 1 **sB**, 2 **sb**. It is clear, however, that

TABLE IV.

Single coupling $\begin{array}{c}\mathbf{XY}\\ \mathbf{xy}\\ \mathbf{xy}\\ \mathbf{xy}\end{array}$

Parents*		No. of families	**SB**	**Sb**	**sB**	**sb**
$\mathbf{SB}.(\mathbf{sb})_3\times(\mathbf{sb})_4$	(*a*)	35	475	59	45	453
,, ,,	(*b*)	19	191	21	12	148
,, ,,	(*c*)	2	101	6	11	83
,, ,,	(Total)	56	767	86	68	684
,, ,,	(Calc.)	—	738·2	64·3	64·3	738·2
$(\mathbf{sb})_4\times\mathbf{SB}.(\mathbf{sb})_3$	(*a*)	24	229	25	23	303
,, ,,	(*b*)	15	88	9	8	72
,, ,,	(*c*)	2	13	1	0	14
,, ,,	(Total)	41	330	35	31	389
,, ,,	(Calc.)	—	359·5	33	33	359·5
$\mathbf{SB}.(\mathbf{sb})_3\times\mathbf{SB}.(\mathbf{sb})_3$	(*a*)	14	304	18	17	96
,, ,,	(*b*)	8	145	7	8	53
,, ,,	(*c*)	7	137	4	9	40
,, ,,	(Total)	29	586	29	34	189
,, ,,	(Calc.)	—	595·5	33·0	33·0	176·5
			SG	**Sg**	**sG**	**sg**
$\mathbf{SG}.(\mathbf{sg})_3\times(\mathbf{sg})_4$	(*a*)	15	178	114	101	169
,, ,,	(*b*)	2	26	12	12	24
,, ,,	(Total)	17	204	126	113	193
,, ,,	(Calc.)	—	198·5	119·5	119·5	198·5
$(\mathbf{sg})_4\times\mathbf{SG}.(\mathbf{sg})_3$	(*a*)	16	124	80	95	155
,, ,,	(*b*)	4	8	9	2	5
,, ,,	(Total)	20	132	89	97	160
,, ,,	(Calc.)	—	146	93	93	146
$\mathbf{SG}.(\mathbf{sg})_3\times\mathbf{SG}.(\mathbf{sg})_3$	(*a*)	7	179	47	40	27
,, ,,	(Calc.)	—	174·4	45·3	45·3	27·9
			BG	**Bg**	**bG**	**bg**
$\mathbf{BG}.(\mathbf{bg})_3\times(\mathbf{bg})_4$	(*a*)	16	190	105	93	180
,, ,,	(*b*)	4	33	16	21	30
,, ,,	(Total)	20	223	121	114	210
,, ,,	(Calc.)	—	216·5	117·5	117·5	216·5
$(\mathbf{bg})_4\times\mathbf{BG}.(\mathbf{bg})_3$	(*a*)	16	135	69	84	165
,, ,,	(*b*)	4	8	9	2	5
,, ,,	(Total)	20	143	78	86	170
,, ,,	(Calc.)	—	156·5	82	82	156·5
$\mathbf{BG}.(\mathbf{bg})_3\times\mathbf{BG}.(\mathbf{bg})_3$	(*a*)	7	177	48	42	26
,, ,,	(*b*)	2	14	1	4	2
,, ,,	(Total)	9	191	49	46	28
,, ,,	(Calc.)	—	190·4	45·1	45·1	33·4

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* Throughout these tables the parent used as a female is put first. The groups in which the parents are of the same composition almost all arise from self-fertilisation.

some anomaly is occurring here (possibly a mistake was made in crossing). The chance of obtaining such a family by random sampling is much less than one in a billion. It has therefore been omitted from the total figures used in calculating the **SB** cross-over value of 8·01 per cent. Its inclusion would bring this value up to 9·53 per cent. Another plant 180^3/28, believed from its ancestry to be $\mathbf{SBG.(sbg)_3}$, when used as a female with $\mathbf{(sbg)_4}$ gave normal coupling of **S** and **B**, but 7 **SG**, 10 **Sg**, 9 **sG**, 6 **sg** and 6**BG**, 10 **Bg**, 10 **bG**, 6**bg**, as if **G** were being repelled from **S** and **B**. As male and when selfed the numbers were too small to be decisive. However, three **SBG** plants from the anomalous family 70–73/29 whose numbers are given

TABLE V.

Single repulsion **Xy**
xY
xy
xy

Parents		No. of families	SB	Sb	sB	sb
$\mathbf{Sb.sB.(sb)_2\times(sb)_4}$	(*a*)	3	36	43	39	28
,, ,,	(*c*)	1	8	12	6	6
,, ,,	(Total)	4	44	55	45	34
,, ,,	(Calc.)	—	32	57	57	32
$\mathbf{(sb)_4\times Sb.sB.(sb)_2}$	(*a*)	3	11	25	15	15
,, ,,	(Calc.)	—	11·9	21·1	21·1	11·9
$\mathbf{Sb.sB.(sb)_2\times Sb.sB.(sb)_2}$	(*b*)	2	40	17	11	0
,, ,,	(Calc.)	—	36·2	14·8	14·8	2·2
			SG	**Sg**	**sG**	**sg**
$\mathbf{Sg.sG.(sg)_2\times(sg)_4}$	(*a*)	11	72	76	77	61
,, ,,	(*b*)	12	54	60	69	41
,, ,,	(Total)	23	126	136	146	102
,, ,,	(Calc.)	—	116·9	138·1	138·1	116·9
$\mathbf{(sg)_4\times Sg.sG.(sg)_2}$	(*a*)	9	31	43	46	40
,, ,,	(*b*)	5	7	14	14	12
,, ,,	(Total)	14	38	57	60	52
,, ,,	(Calc.)	—	47·9	55·6	55·6	47·9
			BG	**Bg**	**bG**	**bg**
$\mathbf{Bg.bG.(bg)_2\times(bg)_4}$	(*a*)	7	32	32	38	31
,, ,,	(*b*)	10	47	48	62	37
,, ,,	(Total)	17	79	80	100	68
,, ,,	(Calc.)	—	73·7	89·8	89·8	73·7
$\mathbf{(bg)_4\times Bg.bG.(bg)_2}$	(*a*)	4	10	22	30	24
,, ,,	(*b*)	5	6	14	15	12
,, ,,	(Total)	9	16	36	45	36
,, ,,	(Calc.)	—	29·8	36·7	36·7	29·8
$\mathbf{Bg.bG.(bg)_2\times Bg.bG.(bg)_2}$	(*c*)	1	8	5	4	1
,, ,,	(Calc.)	—	9·9	3·6	3·6	0·9

above were selfed, and all behaved as if **SBG** were coupled, the totals being 29 **SG**, 10 **Sg**, 17 **sG**, 7 **sg**. The family 70–73/29 is therefore included, although the proportion of cross-overs between **B** and **G** deviates by more than six times the standard error of sampling. It must be realised, however, that cross-over values probably do vary in reality, and not only as a result of sampling.

The calculated figures are obtained directly from the back-cross data, so that the agreement in the case of back-crosses is no proof of the correctness of the theory. The agreement is, however, quite satisfactory in the case of the families due to selfing. The inclusion of class (*c*) plants only alters the **SB** cross-over values from 7·93 to 8·01, and from 8·59 to 8·41 per cent.

The data for single repulsion are given in Table V. The figures are decidedly irregular, but this is mainly due to the single factor ratios. If there is any systematic difference between observed and calculated linkage, it should show up in a difference between observed and calculated numbers of **XY** + **xy** zygotes (*i.e.* **SB** + **sb**, etc.) in the crosses giving 1 : 1 single factor ratios. The observed numbers are 621 (**XY** + **xy**), 800 (**Xy** + **xY**), the calculated 624·4 and 796·6. The agreement is thus very good, and the theory as a whole is confirmed. It is at once clear that the phenomenon is quite different from repulsion in a diploid.

In the case of asymmetrical coupling and repulsion a number of families have not been included which illustrate linkage of **G** with **S** and **B**. These are class (*c*) families. That is to say, the type of linkage is deduced from the family concerned, and is not certain from the ancestry. In the case of linkage between **S** and **B**, however, such families are included. The linkage being strong, there is little chance of mistaking coupling for repulsion, especially when the plant whose composition is doubtful has been both crossed and selfed. Except in the case of the asymmetrical repulsion of **S** and **G**, which arose from frequent crosses between horticultural varieties homozygous for **S** and **G**, and $\mathbf{Ss_3g_4}$ plants, the data are scrappy, and it is difficult to be sure how far the disagreements of theory and observation are fortuitous.

Summing the asymmetrical coupling figures from

$$\mathbf{XY\,.\,Xy\,.\,(xy)_2 \times (xy)_4}$$

and the reciprocal we have:

	XY	**Xy**	**xY**	**xy**
Found	598	431	33	138
Calculated	544·5	455·9	55·9	144·1

TABLE VI.

Asymmetrical coupling $\begin{matrix}\mathbf{XY}\\ \mathbf{Xy}\\ \mathbf{xy}\\ \mathbf{xy}\end{matrix}$

Parents		No. of families	**SB**	**Sb**	**sB**	**sb**
$\mathbf{SB.Sb.(sb)_2\times(sb)_4}$	(*b*)	1	6	12	1	1
,, ,,	(*c*)	7	49	32	0	11
,, ,,	(Total)	8	55	44	1	12
,, ,,	(Calc.)	—	54·5	38·8	1·5	17·2
$\mathbf{SB.sB.(sb)_2\times(sb)_4}$	(*b*)	8	100	1	51	18
,, ,,	(*c*)	1	18	0	13	11
,, ,,	(Total)	9	118	1	64	29
,, ,,	(Calc.)	—	103·2	2·8	73·5	32·5
$\mathbf{(sb)_4\times SB.Sb.(sb)_2}$	(*c*)	6	22	11	0	6
,, ,,	(Calc.)	—	18·9	13·6	0·55	5·9
$\mathbf{(sb)_4\times SB.sB.(sb)_2}$	(*c*)	1	2	0	2	0
,, ,,	(Calc.)	—	2·1	0·05	1·4	0·6
$\mathbf{SB.Sb.(sb)_2\times SB.Sb.(sb)_2}$	(*b*)	3	33	14	1	1
,, ,,	(*c*)	1	2	1	0	0
,, ,,	(Total)	4	35	15	1	1
,, ,,	(Calc.)	—	38·8	11·8	0·2	1·2
$\mathbf{SB.sB.(sb)_2\times SB.sB.(sb)_2}$	(*b*)	5	71	1	32	0
,, ,,	(Calc.)	—	77·5	0·5	23·6	2·4
			SG	**Sg**	**sG**	**sg**
$\mathbf{SG.Sg.(sg)_2\times(sg)_4}$	(*b*)	3	26	15	3	5
,, ,,	(Calc.)	—	21·4	19·4	3·1	5·1
$\mathbf{SG.sG.(sg)_2\times(sg)_4}$	(*a*)	1	3	0	4	2
,, ,,	(*b*)	13	153	9	104	33
,, ,,	(Total)	14	156	9	108	35
,, ,,	(Calc.)	—	134·7	19·3	122	32
$\mathbf{(sg)_4\times SG.sG.(sg)_2}$	(*a*)	1	12	5	20	7
,, ,,	(*b*)	4	36	2	30	6
,, ,,	(Total)	5	48	7	50	13
,, ,,	(Calc.)	—	51·4	7·7	47	12
$\mathbf{SG.sG.(sg)_2\times SG.sG.(sg)_2}$	(*a*)	3	101	1	30	2
,, ,,	(*b*)	7	272	6	88	8
,, ,,	(Total)	10	373	7	118	10
,, ,,	(Calc.)	—	371·2	9·8	121·6	5·4
			BG	**Bg**	**bG**	**bg**
$\mathbf{BG.bG.(bg)_2\times(bg)_4}$	(*b*)	10	122	7	88	23
,, ,,	(Calc.)	—	105·9	14·1	94·1	25·9
$\mathbf{(bg)_4\times BG.bG.(bg)_2}$	(*a*)	1	12	3	20	9
,, ,,	(*b*)	4	37	2	29	6
,, ,,	(Total)	5	49	5	49	15
,, ,,	(Calc.)	—	52·2	6·8	46·1	12·9
$\mathbf{BG.bG.(bg)_2\times BG.bG.(bg)_2}$	(*b*)	8	175	5	70	4
,, ,,	(Calc.)	—	186·4	4·1	60·5	3

The disagreement in the case of the small cross-over class **xY** is serious. But it is partly due to the very bad single factor ratio for **X**, the number of recessives being only 171 instead of 200. Making allowance for this the number of **xY** would be increased to 39·3. The divergence is now only just over twice the standard error, and not certainly significant. Only further work can decide whether the theory holds in this case. On the other hand in the case of selfed plants we have:

	XY	**Xy**	**xY**	**xy**
Found	654	235	14	15
Calculated	675·9	218·6	13·5	12·0

The agreement is much better, and the cross-overs are in excess of expectation, which suggests that the disagreement in the former case is due to bad luck.

In the case of asymmetrical repulsion, theory agrees very well with observation. The figures could be used to calculate linkage intensity. Thus if, the expectation being $3 - p : 2 + p : 1 - p : p$, the numbers found are a, b, c and d, the method of maximum likelihood (cf. Fisher and Balmukand (1928)) shows that p is a root of

$$\frac{a}{p-3} + \frac{b}{p+2} + \frac{c}{p-1} + \frac{d}{p} = 0,$$

or

$$(a + b + c + d)\,p^3 - (-a + 4b + c + 2d)\,p^2$$
$$-(2a - 3b + 6c + 5d)\,p + 6d = 0.$$

Applying this equation to the data of $\mathbf{Sg}.(\mathbf{sG})_2.\mathbf{sg} \times (\mathbf{sg})_4$, where $a = 439$, $b = 422$, $c = 98$, $d = 70$, we have:

$$1029p^3 - 1487p^2 - 550p + 420 = 0,$$

whence $p = 42{\cdot}2$ per cent. as compared with 37·6 per cent. from the single coupling data. We have not however used such figures to correct the linkage values used, since the agreement of observation and calculation is more logically demonstrated when the latter is based on single coupling only.

The majority of figures for double coupling of **S** and **B** come from a few plants which are placed in class (c) but whose composition is not really in the least doubt. They were derived from the self-fertilisation of known $\mathbf{SB}.(\mathbf{sb})_3$ plants, and have given large families which make it clear that they are of the composition $(\mathbf{SB})_2.(\mathbf{sb})_2$. Actually of the $\mathbf{S}_2\mathbf{s}_2\mathbf{B}_2\mathbf{b}_2$ plants from such ancestry 84 per cent. should be of the above composition, and the remainder should give many more **Sb** and **sB** than **sb** plants when crossed to a recessive. The progeny of two of these plants is also included in the tables for double coupling of **G** with **S** and **B**, but the

TABLE VII.

Asymmetrical repulsion $\begin{matrix}\mathbf{Xy}\\ \mathbf{Xy}\\ \mathbf{xY}\\ \mathbf{xy}\end{matrix}$

Parents		No. of families	**SG**	**Sg**	**sG**	**sg**
$\mathbf{Sg.(sG)_2.sg \times (sg)_4}$	(*a*)	25	260	64	297	45
,, ,,	(*b*)	19	162	34	142	25
,, ,,	(Total)	44	422	98	439	70
,, ,,	(Calc.)	—	407·5	107	450	64·5
$\mathbf{(sg)_4 \times Sg.(sG)_2.sg}$	(*a*)	8	24	6	31	4
,, ,,	(*b*)	2	9	1	7	0
,, ,,	(Total)	10	33	7	38	4
,, ,,	(Calc.)	—	32·7	8·3	35·7	5·3
$\mathbf{Sg.(sG)_2.sg \times Sg.(sG)_2.sg}$	(*a*)	12	520	25	152	5
,, ,,	(*b*)	13	327	6	115	2
,, ,,	(Total)	25	847	31	267	7
,, ,,	(Calc.)	—	836·8	27·2	283·2	4·8
			BG	**Bg**	**bG**	**bg**
$\mathbf{Bg.(bG)_2.bg \times (bg)_4}$	(*b*)	3	39	8	39	10
,, ,,	(Calc.)	—	37·5	10·4	42·5	5·6
$\mathbf{(bg)_4 \times Bg.(bG)_2.bg}$	(*b*)	4	13	2	8	0
,, ,,	(Calc.)	—	9	2·5	10·2	1·3
$\mathbf{(Bg)_2.bG.bg \times (Bg)_2.bG.bg}$	(*b*)	2	69	32	3	1
,, ,,	(Calc.)	—	76·6	25·9	2·1	0·35
$\mathbf{Bg.(bG)_2.bg \times Bg.(bG)_2.bg}$	(*a*)	1	15	3	9	1
,, ,,	(*b*)	3	24	1	9	0
,, ,,	(Total)	4	39	4	18	1
,, ,,	(Calc.)	—	45	1·5	15·3	0·21

propriety of this step is less certain. The total number of cross-overs, 118, is less than the expectation, 134·2, but not sufficiently so to warrant the deduction that crossing-over between one pair of chromosomes hinders simultaneous crossing-over between the other pair. Certainly, however, there is no suggestion of a positive correlation between the two processes.

The double repulsion figures are less satisfactory. Nevertheless the class (*b*) families demonstrate the existence of the phenomenon in the case of **B** and **G**. Some of the class (*c*) families here included may really be examples of the seventh type of linkage, viz. coupling with repulsion. However, in each case considerations favour the assignment here given. There is possibly, as in the last case, a deficiency of the zygotic type (here the double recessive) which is due to simultaneous crossing-over. Besides the families of Tables VIII and IX a large number of other families are on record which are derived from parents duplex for two factors. But the evidence regarding their linkage is quite inconclusive. They may in most cases be examples either of double coupling, of double repulsion or of coupling and repulsion. Unfortunately we have as yet no clear case of the latter type of linkage.

We are aware that the data on double coupling and repulsion are unsatisfactory. In order to remedy this defect it is proposed to establish pure (*i.e.* quadruplex) lines of dominants. Since, however, two generations are required to test the homozygosity of such lines, satisfactory data will not be available for some years, and it has been thought best to publish the present evidence, which clearly demonstrates the existence of double coupling and repulsion, although the precise laws which they obey are still in some doubt.

TABLE VIII.

Double coupling $\begin{matrix}\mathbf{XY}\\ \mathbf{XY}\\ \mathbf{xy}\\ \mathbf{xy}\end{matrix}$

Parents		No. of families	**SB**	**Sb**	**sB**	**sb**
$(\mathbf{SB})_2.(\mathbf{sb})_2 \times (\mathbf{sb})_4$	(*b*)	1	14	1	0	0
,, ,,	(*c*)	7	274	7	4	54
,, ,,	(Total)	8	288	8	4	54
,, ,,	(Calc.)	—	285·9	9·1	9·1	49·9
$(\mathbf{sb})_4 \times (\mathbf{SB})_2.(\mathbf{sb})_2$	(*c*)	2	84	5	3	16
,, ,,	(Calc.)	—	87·1	2·9	2·9	15·1
$(\mathbf{SB})_2.(\mathbf{sb})_2 \times (\mathbf{SB})_2.(\mathbf{sb})_2$	(*c*)	5	146	1	0	3
,, ,,	(Calc.)	—	144·6	1·2	1·2	3·0
			SG	**Sg**	**sG**	**sg**
$(\mathbf{SG})_2.(\mathbf{sg})_2 \times (\mathbf{sg})_4$	(*b*)	3	16	0	3	1
,, ,,	(*c*)	3	130	18	10	17
,, ,,	(Total)	6	146	18	13	18
,, ,,	(Calc.)	—	142·7	19·8	19·8	12·7
$(\mathbf{sg})_4 \times (\mathbf{SG})_2.(\mathbf{sg})_2$	(*b*)	1	45	6	3	11
,, ,,	(*c*)	1	36	7	6	6
,, ,,	(Total)	2	81	13	9	17
,, ,,	(Calc.)	—	87·5	12·5	12·5	7·5
$(\mathbf{SG})_2.(\mathbf{sg})_2 \times (\mathbf{SG})_2.(\mathbf{sg})_2$	(*b*)	3	80	2	2	2
,, ,,	(*c*)	1	24	1	2	0
,, ,,	(Total)	4	104	3	4	2
,, ,,	(Calc.)	—	107·2	2·7	2·7	0·45
			BG	**Bg**	**bG**	**bg**
$(\mathbf{BG})_2.(\mathbf{bg})_2 \times (\mathbf{bg})_4$	(*c*)	1	101	13	9	13
,, ,,	(Calc.)	—	100·2	13·1	13·1	9·5
$(\mathbf{bg})_4 \times (\mathbf{BG})_2.(\mathbf{bg})_2$	(*c*)	1	36	7	6	6
,, ,,	(Calc.)	—	40·6	5·2	5·2	3·9
$(\mathbf{BG})_2.(\mathbf{bg})_2 \times (\mathbf{BG})_2.(\mathbf{bg})_2$	(*c*)	1	24	0	2	1
,, ,,	(Calc.)	—	25·6	0·61	0·61	0·14

TABLE IX.

Double repulsion **Xy**
Xy
xY
xY

Parents		No. of families	**SG**	**Sg**	**sG**	**sg**
$(\mathbf{Sg})_2 . (\mathbf{sG})_2 \times (\mathbf{sg})_4$	(*c*)	12	124	21	19	0
,, ,,	(Calc.)	—	113·3	23·4	23·4	3·9
$(\mathbf{sg})_4 \times (\mathbf{Sg})_2 . (\mathbf{sG})_2$	(*c*)	2	11	0	1	0
,, ,,	(Calc.)	—	8·3	1·7	1·7	0·30
			BG	**Bg**	**bG**	**bg**
$(\mathbf{Bg})_2 . (\mathbf{bG})_2 \times (\mathbf{bg})_4$	(*b*)	5	110	24	14	2
,, ,,	(*c*)	3	40	14	8	0
,, ,,	(Total)	8	150	38	22	2
,, ,,	(Calc.)	—	145·7	31·0	31·0	4·4
$(\mathbf{bg})_4 \times (\mathbf{Bg})_2 . (\mathbf{bG})_2$	(*b*)	1	4	0	0	0
,, ,,	(Calc.)	—	2·7	0·54	0·54	0·08
$(\mathbf{Bg})_2 . (\mathbf{bG})_2 \times (\mathbf{Bg})_2 . (\mathbf{bG})_2$	(*b*)	5	102	1	1	0
,, ,,	(Calc.)	—	98·2	2·8	2·8	0·04

Linkages between three factors.

The totals of 30 families, all of class (*a*), in which all three factors were singly coupled, are collected in Table X. In 15 the cross was

$$\mathbf{SBG} . (\mathbf{sbg})_3 \times (\mathbf{sbg})_4,$$

in the other 15 $(\mathbf{sbg})_4 \times \mathbf{SBG} . (\mathbf{sbg})_3$.

The ratios expected are the same as in diploid linkage. The expectations given in the table are calculated from the linkage values found for the factors two at a time. The agreement found merely shows that the families considered are a fair sample.

The interest centres on the double cross-overs, which are fewer than expected. If there were no interference, *i.e.* if crossing-over between **S** and **B** did not diminish the probability of crossing-over between **B** and **G**, the expected values in these families would be $\frac{41 \times 183}{543}$, or 13·8 on the female side, and $\frac{29 \times 147}{420}$, or 10·15 on the male side. The average coincidence is thus 67 per cent., most marked on the male side. On the basis of the cross-over values of Table I the coincidence is 99 per cent. on the female side, and 69 per cent. on the male side. But these estimates are less reliable because the figures used are not all drawn from the same families. In the diploid the data of Gregory, de Winton and Bateson give a coincidence of 89 per cent. on the female side and 83 per cent. on the male side. The concordance is quite satisfactory in view of the small numbers. There is no suggestion that double crossing-over is easier in the tetraploid owing to exchanges involving three chromosomes.

TABLE X.

Progeny of **SBG**.$(\mathbf{sbg})_3$ × $(\mathbf{sbg})_4$ *and reciprocally.*

		Ex Het. ♀		Ex Het. ♂	
SBG	———	173	330 (323·7)	112	249 (248·6)
sbg		157		137	
Sbg	———	15	30 (28·3)	11	24 (27·0)
sBG		15		13	
SBg	———	92	172 (175·8)	66	142 (136·0)
sbG		80		76	
SbG	———	5	11 (15·2)	2	5 (8·4)
sBg		6		3	

TABLE XI.

SBG.$(\mathbf{sbg})_3$ *selfed. Six families.*

	SBG	**SBg**	**SbG**	**Sbg**	**sBG**	**sBg**	**sbG**	**sbg**
Found	169	45	10	2	8	3	32	24
Calculated	169·2	39	5·2	6·3	8·4	3·1	36·9	24·8

TABLE XII.

Sbg.**sBG**.$(\mathbf{sbg})_2$ × $(\mathbf{sbg})_4$ *and reciprocally.*

SBG	14	25 ($23{\cdot}5 = 1 + x - y - z$)
sbg	11	
SBg	10	20 ($12{\cdot}2 = y + 2z$)
sbG	10	
SbG	10	24 ($22{\cdot}1 = 2y + z$)
sBg	14	
Sbg	18	30 ($41{\cdot}2 = 2 - x - 2y - 2z$)
sBG	12	

In a diploid, satisfactory data regarding the linkage of three factors can be obtained even when one is repelled from the other two. This is not so in a tetraploid. Four class (*a*) families are derived from the mating **Sbg**.**sBG**.$(\mathbf{sbg})_2$ × $(\mathbf{sbg})_4$, and from the reciprocal cross. They are summarised in Table XII. The expectation is calculated on the basis that x is the expected proportion of cross-overs between the loci of **S** and **B**, y between those of **B** and **G**, and z the proportion of double cross-overs. The values taken, weighted to allow for the fact that reciprocal crosses are added together, are $x = 0{\cdot}0582$, $y = 0{\cdot}3220$, $z = 0{\cdot}0243$. It will be seen that the agreement of theory and expectation is poor. But it is also clear that such data would be useless for calculating z. Other families exist in which **S**, **B** and **G** were all in different chromosomes. But they are mostly in class (*b*), and cannot be expected to agree very well with theory; nor do they throw any light on double crossing-over.

In Table XI are collected six families, all of class (a), from the selfing of **SBG**.(**sbg**)$_3$ plants. The agreement with theory is on the whole good. The only class which is necessarily due to double crossing-over of one chromosome is **sBg**. **SbG** can of course be formed by one cross-over on the female side and another on the male side.

DISCUSSION.

It is at once clear that the various results obtained agree fairly well with expectation on the chromosome theory. In fact the agreement is rather surprising in view of the numerous irregularities in meiosis which Darlington (1930) has described in this plant. We should have expected to find single factor ratios nearer to those deduced from a basis of random segregation between chromatids, and also evidence of crossing-over involving three chromosomes, double reduction, and other anomalies. The regularity observed may be due to several causes. The chromosome considered may have little tendency to form quadrivalents at meiosis. This is rather unlikely, as it contains 6 of the 27 known factors, and is therefore probably fairly long.

A somewhat more likely view, suggested to me by Dr Darlington, is that the three factors in question are located rather near the attachment constriction of the chromosome in which they are situated. In this case segregation would be little affected by the fact that pairing is between chromatids, and not whole chromosomes. The only common type of non-disjunction to be expected in such a case would be that leading to $2n + 1$ or $2n - 1$ gametes, which are doubtless largely eliminated, though as pointed out earlier in the paper, it is probable that some of our parent plants possessed an extra chromosome in the set carrying the factors discussed.

Table I gives a comparison of linkage values. It is obvious that the difference in linkage intensity which exists in the diploid between the male and the female sides of the plant is here absent or very slight. In each case the tetraploid values found are intermediate between those found on the two sides of the diploid. The differences, however, are not always large compared with their standard errors; but that between the cross-over values for **S** and **B** on the male side is 4·50 per cent., with a standard error of only 1·11 per cent.; the difference being four times its standard error, the odds in favour of its significance are nearly 10,000 to 1, assuming the errors to be due to sampling only. Even though, as pointed out above, a few families are aberrant, so that errors are not solely due to sampling, the difference is probably real.

The work is being continued. Recent observations, both on the diploid and tetraploid, have made it probable that the factor **V**, in whose absence the stem is green, is linked with **S**, **B** and **G**. We hope to compare this linkage in the diploid and tetraploid. The linked factors **F** (for flat as opposed to crimped leaves) and **Ch** (for *sinensis* as opposed to *stellata* type) are also available in the tetraploid. But the dominance of both is so incomplete as to render them unsuitable for accurate work. Before the theory here given can be regarded as generally applicable to autotetraploids it is desirable that it should be tested on other plants. Dr Sansome is at present engaged in a study of linkage in the tetraploid tomato at this Institution.

SUMMARY.

1. An account is given of six types of linkage observed between three pairs of factors in the tetraploid *Primula sinensis*, and of a seventh theoretically possible type.

2. The intensity of linkage is nearly, but not quite, the same in the tetraploid as in the diploid. It is the same on the two sides in the tetraploid.

3. As regards the factors considered, there is no evidence of crossing-over involving more than two chromosomes at a time, or of two chromosomes going to the same pole after crossing-over.

4. The six readily available gametic series contain only one adjustable constant p, and since the experimental results in other cases agree reasonably well with prediction when p has been calculated from the results of single coupling, this affords substantial support of the chromosome theory of inheritance.

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