NON-DISJUNCTION AS PROOF OF THE CHROMOSOME THEORY OF HEREDITY (concluded)

CALVIN B. BRIDGES

Columbia University, New York City

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CYTOLOGICAL PROOF OF THE OCCURRENCE OF XXY FEMALES

The breeding work presented in the last two sections has furnished data which show that the cause of secondary non-disjunction cannot be a gene carried by the X chromosome, while the same data are consistent with the assumption that a female which produces secondary exceptions does so because of the presence of an extra Y chromosome. Likewise the data from the tests of the constitution of the regular daughters, the regular sons, the exceptional daughters, and the exceptional sons, all lead to this same conclusion.

Accordingly, the prediction was made that cytological examination of the daughters of an exceptional female would demonstrate the presence of an extra chromosome in half of the daughters while the other half

would show only normal figures. This prediction has been completely verified.

The ovaries at the mid-pupal stage of development offer the best material for examination. Nearly a hundred pairs of ovaries were dissected from pupae of cultures the mother of which was an exceptional female. Eighteen of these gave oögonial metaphases which were clear enough to give a reliable count of the chromosomes.

Nine of the females showed figures like those published by Miss STEVENS (1908 a) in which the X chromosomes are a pair of straight rods. (See plate 1, figures 1-3, as well as the generalized group in figure 1, p. 3 of the first instalment).

The other nine females showed these two X chromosomes and in addition a chromosome which differed from both the X's in that it had the shape of a V with one arm shorter than the other. This chromosome was identified as the Y from the following considerations.

No figures which showed this extra chromosome were found by METZ (1914) when he examined the chromosomes of several wild stocks of *Drosophila ampelophila*. From the work of STEVENS (1908 a), METZ (1914) and myself (plate 1, figs. 1-3) there can be no doubt that the normal condition of the female is that shown in figure 1 (p. 3, first instalment.) The new type of figure which I found differs from the normal only by the addition of this chromosome. Fortunately, there are several good figures in each of four or five of the XXY females, and all the figures in any one female show the same condition.

The figures given by STEVENS, METZ, and myself, show that homologous chromosomes usually lie together as actual pairs. In the figures showing the extra chromosome, this chromosome is usually found in company with the two straight chromosomes so that it behaves as a homologue to them.

Very recently I have found several excellent spermatogonial figures in the testes of larval males; these show beyond question that the identification of the Y has been correct, for the Y has in these males the same characteristics as the supernumerary chromosome of the XXY females.

Breeding tests with sex-linked characters have shown that half the regular daughters produce exceptions to the inheritance of sex and sex-linked characters; parallel with this is the fact that half of the regular daughters possess this extra Y chromosome. Normal females do not possess this chromosome and do not produce exceptions, so that the exceptions must be produced by the daughters with the extra chromosome.

Recently over forty freshly hatched females which were first classi-

fied as exceptions were dissected and a cytological examination made of their chromosomes. In over a dozen of these individuals sufficiently clear figures were found to be sure of the number and character of the chromosomes. *In every case the exceptional female was found to be XXY.* This direct examination of the exceptions gives entirely conclusive proof that the cause of the production of secondary exceptions is the presence of the Y.

Miss STEVENS's (1908 a) work upon the male showed a pair of unequal chromosomes in place of the pair of equal straight rods of the female The longer of these two chromosomes seems to have the shape of a J in some of STEVENS's figures. The perpetuation of this longer chromosome in the male line can only be explained if these chromosomes have a causal connection with the differentiation of sex.

Of special interest is the condition shown in figures 20 to 24 (plate 1) which are from a single female with two X and two Y chromosomes, XXYY. This female was from a stock culture in which about half the females were expected to be XXY and half the males XYY. As we have seen, nearly half the eggs of an XXY female are XY, and thirty-three percent of the spermatozoa of an XYY male are XY. In the next generation therefore XXYY females should not be at all rare in such a stock. This female gave an unusual number of good figures, there being ten figures in which the identification of every chromosome is fairly certain.

This female has an additional value as evidence since the increase in the number of chromosomes is more striking, and since the occurrence of such a female gives indirect cytological proof of the occurrence of XYY males.

The ratio between XXY and XX daughters was much more easily determined by breeding tests than by cytological examination; accordingly only enough females were examined to prove that XXY females do actually exist. The proof of this point is beyond question. Also it chanced that the ratio of nine XXY to nine XX females was the equality expected from the breeding tests.

It will be noticed that often the figures show chromosomes split in preparation for the coming division. The difficulty in understanding the figures published by STEVENS disappears if it is assumed that in the male such a split in the long arm of the Y chromosomes appears relatively early while the short arm splits later. With this interpretation, practically every figure given by STEVENS falls into line with the evidence which the XXY females furnish, namely, that Y is the

PLATE I

The figures in this plate were drawn at table level; tube length 160 mm; Zeiss compensating ocular 12X; and Zeiss apochromatic 1.5 mm oil immersion objective, N.A. 1.30. The figures were then enlarged 2 ¼ diameters, and in reproduction were reduced in the ratio 3:2. The resulting magnification is 5,115 diameters.

Figures 1-3 are oögonial plates for wild females. Figures 2 and 3 are from the same cyst; figure 1 is from another individual. These figures are from freshly hatched mature flies; the rest of the figures are from pupae.

Figure 4 is a spermatogonial plate of a wild male (the extra granule is probably of no significance). I now have several good figures from the testes of larvae; four of these are diagrammatic in clearness, and show that the Y has the same character in the male as it has when transferred to the female.

Figures 5-19 are from XXY daughters of an exceptional mother. The plates are oögonial with the exception of 6 and 10 which are from other ovarian cells. Figures 5-10 are from one individual, as are 11-13, 14-16, 17, and 18-19. Figure 5 is of unusual clearness.

Figure 13 shows a typical late prophase in which the greatly elongated chromosomes are arranged about the periphery of the nucleus.

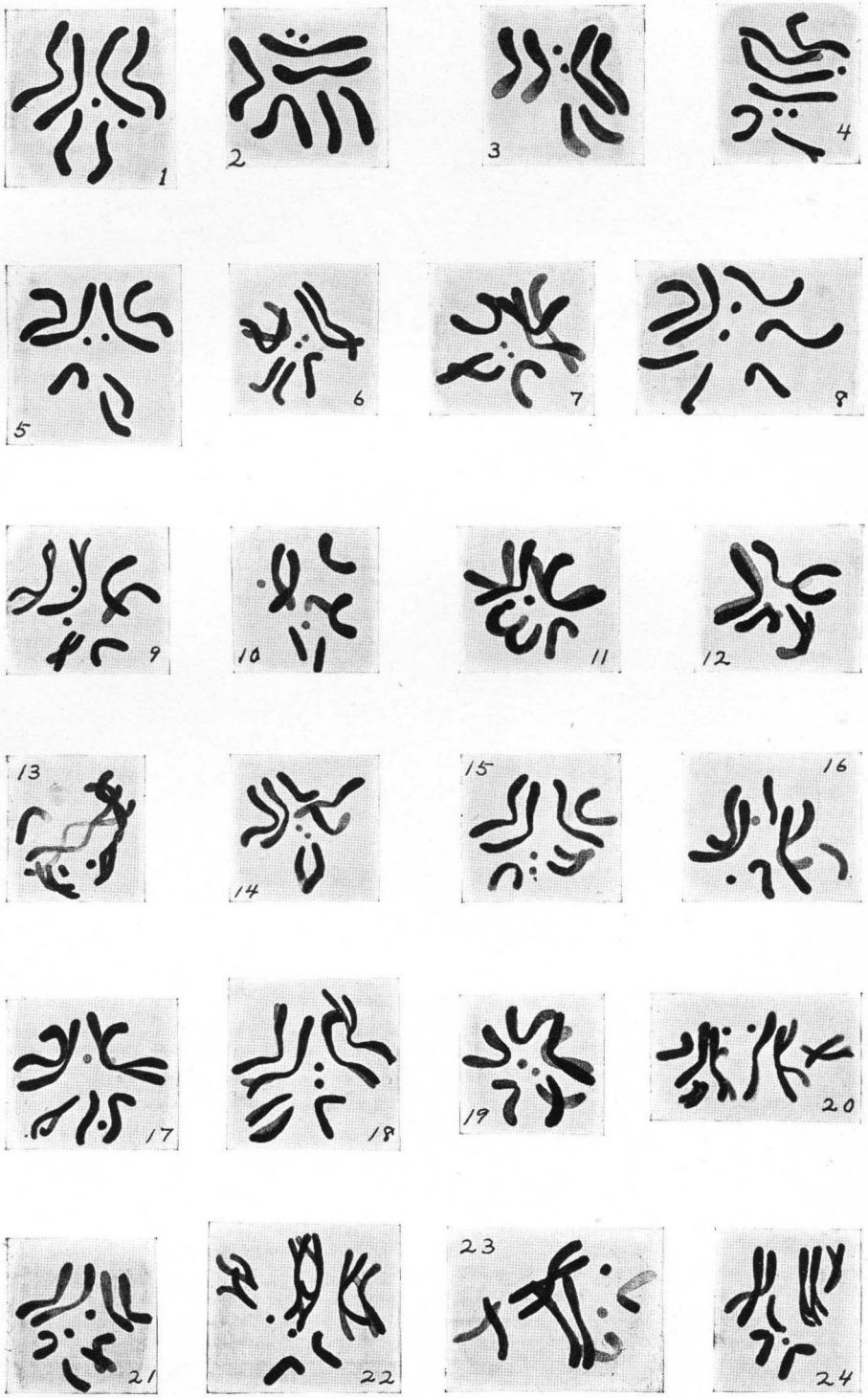
Figure 6 shows the X chromosomes just drawing into the equatorial plate; the outer ends are still curved in contact with the nuclear wall; the same is true of figure 8.

Figures 5, 7, 10-12, 13-16, and 19 are full metaphase groups.

Figure 9 shows a late metaphase group in which the chromosomes are already split; the same condition is seen in 4, 17, 18, and especially well in 20, 22, and 24. It is characteristic of this split that it begins at the free ends of the chromosomes and proceeds toward the spindle attachment; the separation at the point of attachment does not take place until the elongation of the cell.

Figures 20-24 are from an XXYY individual which was found in a stock mass culture in which half the parental flies were XXY females and half were XYY males.

In general the Y is the most sharply defined of all the chromosomes; this is seen especially well in early metaphases such as figure 6, or late metaphases such as figure 22.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longer member of the XY pair, that it is attached sub-terminally, and has a J shape.

THE XX EGGS OF XXY FEMALES

Thus far in our analysis, the XX eggs of an XXY female have been considered mainly in their relation to the production of matroclinous daughters, but the method of origin of these eggs is interesting, if, as so far assumed, they are preceded by synapsis between an X chromosome and a supernumerary Y. In an ordinary XX female, synapsis takes place between X and X, and in an XXY female synapsis must be supposed to follow this same female type in 83.5 percent of cases. During or after synapsis of X with X, crossing over would have an opportunity to occur, and does occur, as will be shown in another section. While the frequency of this crossing over between the two synapsed X's of an XXY female is a little higher than normal the process must be essentially the same as that in an XX female.

But if synapsis in the XXY female should take the course that it follows in the male, where X has Y for its mate, *there would now be no chance of crossing over between the two X's, for one of them is in synapsis with another chromosome, the Y.* At the reduction division, Y and the X which conjugated with it, would pass to opposite poles, and the free X would go either with the Y or with the disjoined X. From those cases where the free X went with the disjoined X, XX eggs and Y eggs would result. The XX eggs should therefore always be *non-crossovers,* and this has been shown to be true.

It was noticed that when an XXY female carried eosin in one of her X chromosomes and vermilion in the other, the exceptional daughters were always like the mother in that they still carried eosin in one X and vermilion in the other. Linkage experiments have shown that about a third of the X eggs of an ordinary XX female are crossovers, that is, they carry both eosin and vermilion in the single X or conversely they carry neither. An experiment was made to find what percentage of XX eggs of an XXY female are crossovers, and the conclusion was reached that none of the XX eggs are crossovers.

If the XXY female is heterozygous for eosin, vermilion and forked instead of for eosin and vermilion only, a greater length of the X can be tested, and over half of the eggs instead of a third are expected to be crossovers under normal conditions. To obtain such females, wild type exceptional daughters (from table 37) were outcrossed to eosin vermilion forked males. The results are given in table 42.

TABLE 42

The offspring given by two wild type exceptional daughters from table 37(A) when outcrossed to eosin vermilion forked males.

No.	All daughters				Regular sons								Exceptions		Percent
	w^e v		w^e | v		w^e t v		w^e | t v		w^e t | v		w^e | t | v				
	w^ev +		w^e v		w^etv +		w^e tv		w^et v		w^ev t		w^evf ♂		
1030	48	48	18	20	48	53	10	20	3	3	—	1	6		4.2
1023	22	15	2	5	12	15	7	7	1	2	—	—	3		6.4
Total	70	63	20	25	60	68	17	27	4	5	—	1	9		

The wild type daughters of table 42 are heterozygous for eosin, vermilion and forked (regular daughters of which carry a Y chromosome, or they are rarely exceptions like their mother). Twelve of these daughters were mated to bar males, with the results shown in table 43.

TABLE 43

A. Regular wild type daughters from table 42 tested by bar males.

No.	Regular daughters	Regular sons								Exceptions		Percent of exceptions
		w^e v f		w^e | v f		w^e v | f		w^e | v | f				
	B′	w^evf +		w^e vf		w^ev f		w^ef v		+ ♀	B′ ♂	
1140	154	32	46	26	19	17	14	1	3	8	7	4.6
1148	74	16	23	4	7	2	4	—	1	4	3	5.1
1122	123	19	24	14	11	13	10	1	—	7	3	4.4
1135	113	25	27	15	20	19	8	4	4	12	5	6.7
1138	125	27	33	18	12	21	16	—	2	12	4	5.9
Total	589	119	153	77	69	72	52	6	10	43	22	5.4
1141	93	31	18	8	16	15	9	3	2	—	—	—
1147	143	29	43	10	16	7	10	4	3	—	—	—
1123	49	22	25	9	10	5	4	1	1	—	—	—
1133	74	26	19	7	6	6	5	1	2	—	—	—
1134	104	13	25	9	12	12	6	2	—	—	—	—
1139	43	12	11	5	5	6	8	—	—	—	—	—
Total	506	133	141	48	65	51	42	11	8	—	—	—

B. Exceptional wild type daughter from table 42 by a bar male.

No.	Regular daughters	Regular sons				Exceptions		Percent of exceptions
		w^e t v	w^e | t v	w^e t | v	w^e | | v | t |			
	B′	w^etv +	w^e tv	w^et v	w^ev t	+ ♀	B′ ♂	
1146	79	24 37	7 12	2 2	— —	—	1	0.6

One of the daughters was herself an exception, as is shown by her offspring (table 43 *B*) which are like the offspring given by her mother. Of the eleven regular daughters five were XXY and six XX (table 43 *A*).

The exceptional daughters from the XXY cultures of table 43 *A*, furnish the material to be tested. Eight such wild type daughters were tested by bar males, and, as shown by their sons, they were all found (table 44) to be non-crossovers, each having eosin, vermilion and forked in one X and only unmutated genes in the homologous X, as their mothers had had.

TABLE 44

The results given by the exceptional wild type daughters from table 43 when tested by bar males.

No.	Regular daughters	Regular sons								Exceptions		Percent of exceptions
		w^e v f		w^e | v f		w^e v | f		w^e | | v | f				
	B′	w^evf +		w^e vf		w^ev f		w^ef v		+ ♀	B′ ♂	
1188	95	24	21	12	14	7	9	3	2	4	1	2.6
1259	125	33	37	15	10	6	8	2	4	8	5	5.1
1197	45	7	7	10	7	2	2	1	—	—	—	—
1186	138	41	53	22	14	12	15	6	—	8	7	4.8
1187	141	44	38	21	11	14	15	1	3	11	4	5.0
1215	115	29	24	12	12	12	10	—	—	8	10	7.8
1216	49	7	13	9	8	6	4	—	—	4	2	5.9
1220	83	20	17	16	11	9	8	1	1	3	7	5.7
Total	791	205	210	117	87	68	71	14	10	46	36	5.0

In subsequent generations twenty-nine more such exceptional daughters have been tested in the same way and all were found to be non-crossovers (table 45).

TABLE 45

The results given by the exceptional wild type daughters from table 44 when tested by bar males.

No.	Regular daughters	Regular sons								Exceptions		
		w^e v f		w^e | v f		w^e v | f		w^e | v | f				
	B′	w^evf	+	w^e	vf	w^ev	f	w^ef	v	+ ♀	B′ ♂	Percent
1400	56	14	10	4	7	3	5	—	—	1	3	3.8
1353	38	8	10	2	4	2	6	—	2	3	—	4.0
1306	65	11	14	11	9	7	3	3	4	2	1	2.3
1332	92	23	23	10	14	6	8	7	1	3	—	1.6
1333	76	15	18	12	11	5	3	5	6	1	2	1.9
1354	18	5	4	1	3	1	—	1	—	—	—	—
1355	47	11	8	10	7	4	4	1	3	1	1	2.1
1334	125	29	39	11	20	17	11	2	1	14	13	9.6
1356	38	12	13	—	6	5	3	—	—	2	1	3.8
1304	99	25	14	10	8	17	7	3	—	5	4	4.7
1357	234	68	85	20	24	28	11	7	2	21	21	8.1
1418	83	16	25	9	15	3	9	4	1	1	1	1.2
1499	29	5	8	5	6	7	5	3	1	2	—	2.8
1500	9	2	1	2	3	1	2	—	—	—	1	4.8
1569	41	7	13	10	5	6	3	1	3	1	3	4.3
1571	71	13	12	12	8	7	8	1	3	3	8	7.5
1572	65	13	12	9	10	7	6	—	—	15	1	11.6
1573	47	11	17	6	6	1	2	1	—	7	—	7.1
1417	63	17	12	2	—	2	8	—	—	22	17	27.3
1503	92	18	28	4	10	8	8	3	—	4	9	7.1
1567	51	23	14	7	4	9	10	—	3	2	2	3.2
1689	56	16	10	5	9	2	2	—	1	3	1	3.8
1612	55	16	14	4	8	5	11	2	—	3	3	5.0
1613	77	15	23	12	10	9	8	2	—	2	4	3.7
1618	170	40	30	13	22	5	13	1	—	14	13	8.4
1624	39	16	9	6	1	4	4	1	—	2	1	3.6
1703	73	12	19	10	11	5	7	1	1	7	8	9.8
1714	188	36	43	28	15	17	17	4	1	10	11	5.7
1715	168	57	48	16	12	13	15	1	2	14	13	7.5(v ♀ 1)
Total	2265	554	576	251	268	206	201	54	36	165	142	6.7

Not one of the thirty-seven exceptional daughters tested was a cross-over, although over twenty of them should have been crossovers if crossing over were of normal frequency. Several other exceptional daughters which were tested in other experiments, increase this evidence and show that crossing over does not occur in the formation of XX eggs. It will be demonstrated later that the XY and X egg of an

XXY female show no decrease in the amount of crossing over. No difference in the way the chromosomes pass to the poles at reduction can explain this difference in the amount of crossing over. The eggs destined to contain XX (or Y) must have begun to differ from those eggs destined to contain XY or X before the reduction division and before crossing over had taken place. This absence of crossing over between the two X's of an XX egg finds a ready explanation on the basis that the two X's were not in synapsis with one another, but that one of them was in heterosynapsis with the supernumerary Y of the XXY female so that crossing over between the two X's was impossible. On page 17 the percentage of cases in which XY synapsis occurs (16.5) was calculated from the percent of exceptions (4.3) given by XXY females.

It has been shown that the two X chromosomes which enter XX eggs are the same two chromosomes that combined in the production of the XXY mother. If these two original chromosomes are X and X′ the two final chromosomes are likewise X and X,′ *but not XX or X′ X′.* This fact proves that the XX-Y separation takes place at the division which in normal eggs would be the *reductional* division, and not at the equational division.

The evidence in this section proves that the ordinary type of non-disjunction is preceded by an XY synapsis and occurs at the reductional division.

SYNAPSIS IN XXY FEMALES

Females of the constitution XXY have been produced in the following three ways, namely, XX egg by Y sperm, XY egg by X sperm, and X egg by XY sperm. Irrespective of their origin, these XXY females have given the same percent of secondary exceptions. This means that the method of synapsis is not influenced by the origin of the chromosomes. Two chromosomes from the *same* parent synapse with each other as readily as though they were from different parents. Thus when both X's come from the mother, they synapse with each other in about 84 percent of cases, and crossing over takes place in about the normal amount. This is of interest in connection with the case of the tetraploid Primula, for as MULLER (1914) has shown, the data of GREGORY (1914) are in accord with the view that the four homologous chromosomes pair with each other, two by two, irrespective of whether they are from the same or from opposite parents.

That synapsis in an XXY female does not involve all three chromosomes at once, but is between two of them with the third chromosome left unsynapsed, is proved by the fact that the chromosomes of XX eggs are never crossovers, while the X's of the X and XY eggs are crossovers in about the usual percent. A difference between the paths followed by these two kinds of chromosomes originated before the stage at which crossing over became possible. If all three chromosomes synapsed together, there should not be this difference, for the X's which enter the XX egg should be crossovers in the same percentage as those which enter the X and XY eggs. Furthermore, it is difficult to see how two X's in synapsis with each other and at the same time with Y can cross over with each other without involving crossing over between the Y and one of the X's. There is no evidence that there is crossing over between X and Y in an XXY female. This leads us to suppose that in the male the lack of crossing over between X and Y is a property of Y and not entirely due to the general fact that there is no crossing over in the male of Drosophila between any chromosomes.

CROSSING OVER IN XXY FEMALES

Besides the production of XX eggs both of whose X's are non-crossovers, there is another consequence of XY synapsis which may be examined. After heterosynapsis Y and the synapsed X disjoin and the free X goes with the Y in half the cases. The resulting XY and X eggs must also be *non-crossovers* since there has been no XX synapsis which would give an opportunity for crossing over to occur. When these eggs are fertilized, they give rise only to regular offspring and should *increase the non-crossover classes of the regular offspring.* Because of this added source of non-crossovers the percentages of crossing over should be *less* than those shown by sister XX cultures. If the free X goes with Y as often as with the disjoined X then the number of these added non-crossover X and XY eggs will be equal to the number of XX and Y eggs. The number of XX and Y eggs is equal to twice the number of surviving exceptions (the XXX and YY zygotes die), so that the number of non-crossovers added from heterosynapsis can be found by doubling the number of observed exceptions. A comparison of the percentages of crossing over shown by XXY and XX cultures should be interesting, and the regular sons of many of the experiments furnishing such data, are summarized in the following tables.

TABLE 45A

A summary of the sons in XXY cultures where three genes were involved.

Genes	—	⊣	⊢	⊢⊣	Total	Exceptional sons	Coincidence	Data from tables
yw^{e}v	491	7	172	2	672	97	86	25
w^{e}tv	1693	606	132	5	2436	65	15	37, 42, 43, 47
w^{e}vf	1890	915	704	142	3651	202	59	43, 44, 45, 63

The first of these tables gives those cases in which crossing over between more than two genes is involved. The data of this table are also included in the following table, which gives the totals for each separate crossover value.

TABLE 45B

A summary of all crossover data from XXY cultures.

Genes	Total sons	Crossovers	Crossover value	Exceptional sons	Corrected value	Data from tables
yw^e	672	9	1.3	97	2.0	25
yv	672	179	26.7	97	37.4	25
w^{e}t	2436	601	24.7	65	26.0	37, 42, 43, 47
w^{e}v	13,014	3937	30.7	580	33.7	9, 12, 23, 25, 35A, 36, 37(*A*), 37(*C*), 42, 43*A*, 43*B*, 44, 45, 47, 56, 63, 77, 86
w^{e}f	3651	1619	44.4	202	49.8	43, 44, 45, 63
w^{e}B′	257	114	44.3	21	53.0	3, 79
tv	2436	137	5.6	65	5.9	37, 42, 43, 47
vf	3651	846	23.2	202	26.0	43, 44, 45, 63

Before discussing these results it is well to summarize the crossing over results given by the XX females. In one case four genes, eosin, vermilion, sable and forked, were run together, and gave the following data.

TABLE 45C

A summary of the sons of XX cultures involving four genes, from tables 34 and 66.

Genes	—	⊣	⊢	⊢	⊢⊣	⊢⊣	⊢⊣	⊢⊣⊣
w^{e}vsf	2708	1213	417	543	55	116	12	0

The two following three-locus cases occurred.

TABLE 45D

A summary of the sons of XX cultures where three genes were involved.

Genes	— — —	— | —	— — |	— | |	Total	Coincidence	Tables
w^{e}tv	1887	627	139	7	2600	19.7	37
w^{e}vf	623	305	223	47	1198	59.4	43, 63

The data from both these tables combined with the other data upon two-locus experiments give the following summary for the offspring of XX cultures.

TABLE 45E

A summary of all crossover data from XX cultures.

Genes	Total	Crossovers	Crossover value	Data from tables
w^{e}t	2600	634	24.4	37
w^{e}v	15,177	4479	29.5	9, 12, 23, 28, 34, 37, 43, 56, 63, 66, 77
w^{e}s	5064	1758	34.7	34, 66
w^{e}f	6262	2701	43.1	34, 43, 63, 66
w^{e}B′	1699	744	43.6	10, 29, 30, 57, 79
tv	2600	146	5.6	37
vs	5064	484	9.6	34, 66
vf	6262	1401	22.4	34, 43, 63, 66
vB′	311	67	21.5	57

On comparing the summary of the results for the XXY cultures with this summary of the results for the XX cultures it is seen that not all of the crossover values occur in both. Those cases in which data of both sorts are present are compared in the following table.

TABLE 45F

A comparison of the crossover values from XX and XXY cultures.

Genes	XX cultures		XXY cultures		Increase	Percentage increase
	Total	Crossover value	Total	Corrected value		
w^{e}t	2600	24.4	2436	26.0	1.6	6.6
w^{e}v	15,177	29.5	12,817	33.7	4.2	14.2
w^{e}f	6262	43.1	3651	49.8	6.7	15.5
w^{e}B′	1699	43.6	257	53.0	9.4	21.6
tv	2600	5.6	2436	5.9	0.3	4.6
vf	6262	22.4	3651	26.0	4.4	19.6

At once the rather startling fact becomes apparent that in every case the percent of crossing over given by the XXY females has risen slightly. The mean increase in the corrected values is 13.5 percent over the values given by the XX cultures. This increase is in some manner due to the presence of the Y in the XXY females, though the mechanism whereby the Y produces such an increase is unknown. The increase does not seem to be confined to one section of the chromosome but seems to be practically uniform throughout the length of the X.

It is possible that some of the assumptions made in the analysis are unwarranted, and that the increase is accordingly only an apparent one. For example, if when X and Y disjoin the free X goes with the disjoined X much more often than with the Y, then only a few non-crossovers would be added to the regular offspring. Some work now under way makes it seem probable that the change in the amount of crossing over is a real one and that it is not necessary to suppose that the assortment at reduction is preferential.

XXX ZYGOTES

By primary non-disjunction in an XX female, and by secondary non-disjunction in an XXY female, XX eggs are produced. We have studied the matroclinous daughters which originate from the fertilization of these eggs by Y sperm; and we have assumed that the equally numerous XXX zygotes produced by the fertilization of XX eggs by X sperm are unable to live. On *a priori* grounds one would expect that XXX zygotes would survive as females. These females might be classified either with the exceptional or with the regular daughters, according to whether the two recessives or the dominant allelomorph produced the greater effect upon the character. More probably the character would be intermediate between the dominant and the recessive, since even in a normal heterozygote there is usually discernible some effect of the recessive. A watch has been kept for such intermediates among the daughters of XXY females, but none have been found. Inspection thus shows that the XXX zygotes do not survive, or else are similar in appearance to the exceptional or to the regular daughters. That they are not among the exceptional daughters is proved by the fact that this class is not more numerous than the exceptional male class, and by the fact that over three hundred of these exceptional daughters have been bred and were all of one type. Likewise about two hundred of the regular daughters have been bred, and none of them gave the peculiar results that an XXX female would be expected to give, namely, a three-female-

to-one-male sex ratio, a ratio of two recessives to one dominant among the sons, a large number of exceptional daughters, and one third of these exceptional daughters of the recessive type. Thus, for example, an XXX female carrying recessive white in two of the X's and the unmutated dominant wild type gene in the other X (w w +) would be wild type in appearance. Bred to a bar male the offspring should be:

Regular daughters	Regular sons	Exceptional daughters	Exceptional sons
6 B′	2w, 1 +	2 +, 1 w	0 B′

If it is still to be supposed that XXX females survive, it must be assumed that they are sterile, since none gave aberrant results among the two hundred regular daughters tested. Only eight or nine would be expected among two hundred regular daughters, and thus the occasionally sterile individual would not seem unusual. While the probability is that XXX zygotes die, this evidence does not yet establish it as a fact.[8]

YY AND YO ZYGOTES

By secondary non-disjunction an XXY female produces as many Y as XX eggs. These Y eggs when fertilized by X sperm give patroclinous sons, but when fertilized by Y sperm give YY zygotes which are unable to live. Such zygotes could not be females for it requires two X chromosomes to develop a female. Every male in a culture shows the sex-linked characters of the mother (regular sons) or of the father (exceptional sons); YY individuals can not be among these males since a YY individual could show no sex-linked characters whatever. By primary non-disjunction eggs with no sex chromosome arise, and these fertilized by Y sperm give YO zygotes which die, as do those with YY.

EQUATIONAL NON-DISJUNCTION

It may now be shown that there is another type of exception which does not preclude XX synapsis, and which occurs at an equational division.

Very rarely a female which is heterozygous for a recessive sex-linked gene produces an exceptional daughter which is pure recessive. Thus a wild type XXY female carrying eosin in one X and vermilion in the other produced an eosin exceptional daughter (culture 600, table 9).

[8] Some work on high non-disjunction now under way proves definitely that XXX zygotes die.

This eosin exception was herself heterozygous for vermilion, as her offspring showed when she was mated by a tan vermilion male (table 46).

TABLE 46

The offspring given by an eosin equational exception from table 9 when tested by a tan vermilion male.

No.	Regular offspring				Exceptions		Percent
	v ♀	+ ♀	w^ev ♂	w^e ♂	w^e ♀	tv ♂	
633	41	52	34	36	4	2	3·5

The presence of the vermilion and the fact that exceptions were produced are both strong indications that no error, such as non-virginity or contamination, occurred. In the case of certain other like exceptions which appeared later there is complete proof of the genuineness of this type of exceptions.

Although in the mother of the eosin exception, eosin was carried in one X and vermilion in the other, in the daughter *one of the X's carried both eosin and vermilion.* There must, then, have been crossing over, preceded by an XX synapsis. The other X of the eosin daughter carried only eosin and therefore was a non-crossover chromosome. The very remarkable case is thus presented of an exceptional daughter, one of whose X chromosomes has undergone crossing over while the other has not!

The chiasmatype hypothesis of crossing over offers a very simple ex-

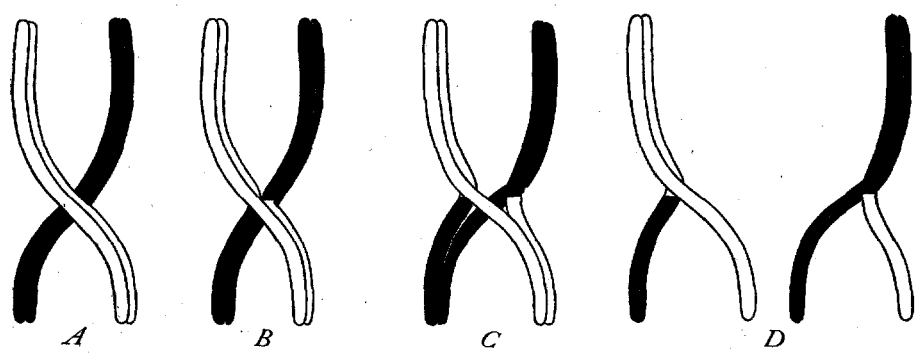


FIGURE 9.—Four stages in crossing over, according to the chiasmatype theory of JANSSENS. The maternal and paternal chromosomes come together, twist about each other, split lengthwise so that four strands result (*A*), and crossing over takes place between only two of the strands (*B*). The reduction division (*C* and *D*) segregates two strands to each cell. (After MORGAN, STURTEVANT, MULLER, and BRIDGES.)

planation of exceptions of this type. In certain known cases, the two X's may come together, twist about each other, and then each split lengthwise, so that four strands are formed. It has been assumed by JANSSENS (1909) that at this stage, where the strands lie in contact there may be crossing over between two without crossing over between the other two. As the strands draw apart each cell should thus receive a crossover and a non-crossover chromosome. If non-disjunction occurred at this stage XX eggs would be formed in which one chromosome is a crossover and the other a non-crossover. Such an egg would give rise to an exceptional daughter of the type actually found.

It is impossible to obtain exceptions of this type from an XX or XXY oöcyte unless the crossing over has taken place at a four strand stage. Therefore the proof that these exceptions have arisen from such oöcytes will at the same time be proof that crossing over takes place during a four strand stage, to which process JANSSENS has given the name chiasmatype.

A few of the offspring of the eosin exceptional female were tested, although there seemed no reason to suppose that they would be in any way different from ordinary XX and XXY flies. One of the wild type daughters tested by yellow white males gave only offspring expected from a regular XXY daughter, as shown by table 47.

TABLE 47

The offspring given by a wild type regular daughter from table 46 when tested by a yellow white male.

No.	Daughters	Regular sons				Exceptions	Percent
	w-w^e +	w^e tv	w^etv +	w^ev +	w^et v	yw ♂	
771	59 80	33 35	24 28	2 5	— —	4	3.1

Three of the eosin exceptional daughters were tested and they proved to be duplicates of the mother; that is, they were heterozygous for vermilion, as expected from their origin through secondary reductional non-disjunction (tables 48, 49 and 50).

TABLE 48

The offspring given by an eosin reduction-exceptional daughter from table 46 when mated by a yellow male.

No.	Regular offspring			Exceptions		Percent
	+ ♀	$w^e v$ ♂	w^e ♂	w^e ♀	y ♂	
750	42	20	26	—	2	2.2

TABLE 49

The offspring given by an eosin reduction-exceptional daughter from table 46 when mated by a yellow white male.

No.	Regular offspring			Exceptions		Percent
	w-w^e ♀	$w^e v$ ♂	w^e ♂	w^e ♀	yw ♀	
800	126	50	67	1	4	2.0

TABLE 50

The offspring given by an eosin reduction-exceptional daughter from table 46 when mated by a vermilion male.

No.	Regular offspring				Exceptions			
	v ♀	+ ♀	$w^e v$ ♂	w^e ♂	w^e ♀	v ♂	Percent	$w^e v$ ♀
712	47	49	51	52	8	11	8.7	1

One of the three eosin daughters (tested by vermilion males, table 50) gave an eosin vermilion exceptional female which is a second case of equational non-disjunction. This immediately suggests that the tendency to produce equational exceptions may be inherited. Rather extensive tests of the offspring were accordingly made.

One of the eosin reductional exceptions tested by pink males gave no equational exceptions (table 51). Pink is an autosomal eye-color (third chromosome) and was used because its presence (heterozygous) in an equational exception would prove beyond question that the intended cross had been made and that no error was responsible for the so-called equational exceptions.

TABLE 51

The offspring given by an eosin reduction-exceptional daughter from table 50 when mated to pink males.

No.	Regular offspring			Exceptions		
	+ ♀	$w^e v$ ♂	w^e ♂	w^e ♀	+ ♂	Percent
772	152	54	62	3	2	1.8

Likewise, four of the regular wild type daughters of table 50 were tested by pink males, but none of these gave equational exceptions (table 52).

TABLE 52

The offspring given by four wild type regular daughters from table 50 when mated by pink males.

No.	All females	All males	Equational female
773	161	166	—
774	162	185	—
775	64	39	—
776	157	170	—
Total	544	560	—

Two of the vermilion regular daughters tested by pink males gave no equational exceptions (table 53).

TABLE 53

The offspring given by two vermilion regular daughters from table 50 when mated by pink males.

No.	Regular offspring			Exceptions		
	+ ♀	$w^e v$ ♂	v ♂	v ♀	+ ♂	Percent
778	144	84	82	1	1	.6
777	156	86	73	—	—	—

Four more vermilion regular daughters tested by forked or by bar males gave no equational exceptions, as is shown in tables 54 and 55.

TABLE 54

The offspring given by a vermilion regular daughter from table 50 *when mated by forked males.*

No.	Regular offspring			Exceptions		
	+ ♀	w^ev ♂	v ♂	v ♀	f ♂	Percent
768	132	64	77	—	—	—

TABLE 55

The offspring given by three vermilion regular daughters from table 50 *when mated by bar males.*

No.	Regular offspring			Exceptions		
	B′ ♀	w^ev ♂	v ♂	v ♀	B′ ♂	Percent
815	175	64	79	4	5	2.8
817	160	70	92	1	3	1.2
Total	335	134	171	5	8	—
816	140	81	87	—	—	—

Finally three of the wild type regular daughters were tested by bar males, with the result that one gave a vermilion daughter which is a third case of equational exception (table 56).

TABLE 56

The offspring given by three wild type regular daughters from table 50 *when tested by bar males.*

No.	Regular daughters	Regular sons				Exceptions			
		w^e / v		w^e | v					
	B′	w^e	v	w^ev	+	+ ♀	B′ ♂	Percent	v ♀
819	143	37	39	18	29	1	4	1.8	—
818	141	58	50	29	17	—	—	—	—
820	171	71	56	33	33	—	—	—	1
Total	312	129	106	62	50	—	—	—	—

In the culture in which the vermilion exception occurred there were no secondary reductional exceptions, the vermilion equational female being the only exception. This absence of other exceptions is not a complete proof that the mother was not XXY; but eight of the regular bar daughters, tested by miniature males (table 57), all failed to give secondary exceptions, which proves conclusively that the mother of the vermilion equational females was XX and that the non-disjunction was primary. It is not remarkable that this third case of equational non-disjunction should have been a primary exception, produced by an XX mother, for the presence of an extra Y cannot explain this type of non-disjunction.

TABLE 57

The offspring given by eight bar regular daughters from table 56 when mated by miniature males.

No.	All daughters	Regular sons				Exceptions
		v — B′		v | B′		
		v	B′	vB′	+	m ♂
976	55	17	23	7	8	—
977	31	17	10	4	1	—
979	47	20	32	5	4	—
1016	115	34	20	6	6	—
1018	129	36	35	15	11	—
Total	377	124	120	37	30	—
	♀ ♀	w^e	B′	w^eB	+	
1017	154	32	33	30	39	—
978	124	124				—
1015	91	106				1 (sterile)

A fourth case of equational exception was an eosin daughter produced by a wild type female heterozygous ("repulsion") for eosin and vermilion (culture 829, table 28). This case also, as has been already shown by the evidence of table 29, was due to primary non-disjunction.

The vermilion equational female recorded in table 56 was outcrossed to eosin males, and showed by her offspring that she was heterozygous for eosin (table 58).

TABLE 58

The offspring given by a vermilion equational daughter from table 56 when tested by an eosin male.

No.	Regular offspring				Exceptions	
	w^{o} ♀	+ ♀	$w^{e}v$ ♂	v ♂	v ♀	w^{e} ♂
938	18	26	24	27	—	—

This exception, like the first, possessed one crossover and one non-crossover chromosome, and suggests the same explanation.

Three of the wild type regular daughters (from table 58) were outcrossed to arc speck males but none of them gave equational exceptions (table 59). Arc and speck are second-chromosome characters which were used to check the result in the same manner as pink was used in the first instance.

TABLE 59

The offspring given by three wild type regular daughters from table 58 when mated by arc speck males.

No.	Females	Males	Equational females
1076	71	73	—
1077	110	94	—
1078	104	91	—
Total	285	258	—

Tests of this same type were applied to a fifth and a sixth case of equational daughters which arose in an unrelated experiment (see pedigree, page 14). Exactly as in the first case, eosin exceptional daughters heterozygous for vermilion arose from mothers which carried eosin in one X and vermilion in the other (cultures 785 and 834, table 23). Each of these eosin daughters was tested by vermilion males and the offspring (table 60) show that they were heterozygous for vermilion.

Several of the wild type daughters from table 60, mated to arc speck males, gave no equational exceptions (table 61).

TABLE 60

The offspring given by two eosin equational daughters from table 23 when mated to vermilion males.

No.	Regular offspring				Exceptions		
	v ♀	+ ♀	w^ev ♂	v ♂	w^e ♀	v ♂	Percent
937	47	58	56	56	2	4	2.7
936	63	57	66	55	5	3	3.2
Total	110	115	122	111	7	7	

TABLE 61

The offspring given by eight wild type regular daughters from table 60 when mated to arc speck males.

No.	Females	Males	Equational females
1072	124	55	—
1073	182	154	—
1074	21	33	—
1075	111	110	—
Total	438	352	—
1068	106	71	—
1069	100	132	—
1070	49	32	—
1071	111	104	—
Total	366	339	—

The second equational female, an eosin vermilion daughter, recorded in table 50, was outcrossed to forked males, and proved to be an XXY female as shown in table 62.

TABLE 62

The offspring given by an eosin vermilion equational female from table 50 when mated to forked males.

No.	Regular offspring		Exceptions		
	+ ♀	w^ev ♂	w^ev ♀	+ ♂	Percent
769	132	111	5	3	3.2

Seven of the wild type regular daughters of table 62 were outcrossed to white bar males. One of these (table 63) gave an eosin vermilion daughter, the seventh equational exception.

TABLE 63

The offspring given by seven wild type regular daughters from table 62 when mated to white bar males.

No.	Regular daughters	Regular sons								Exceptions		
		$\frac{w^e\ v}{\ \ f}$		$\frac{w^e\ \ f}{\ v}$		$\frac{w^e\ v\ f}{\ \ \ }$		$\frac{w^e\ \ \ }{\ v\ f}$				
		$w^e v$	f	$w^e f$	v	$w^e vf$	+	w^e	vf	+♀	wB′♂	Percent
887	102	24	16	13	9	9	11	1	9	1	2	1.5 ($w^e v$♀ 1)
923	67	18	15	5	11	9	5	—	2	1	—	.8
Total	169	42	31	18	20	18	16	1	11	2	2	—
888	105	22	29	12	16	8	10	—	1	—	—	—
889	63	20	18	8	5	4	4	3	1	—	—	—
890	264	69	58	24	46	24	32	10	4	—	—	—
892	160	39	31	13	22	10	10	2	3	—	—	—
922	136	28	35	23	23	16	12	—	4	—	—	—
Total	728	176	171	80	112	62	68	15	13	—	—	—

The eosin vermilion equational exception from table 63 was mated to sable forked males and proved to be heterozygous for forked (table 64). Here again the eosin vermilion forked X was a crossover and the eosin vermilion X a non-crossover.

TABLE 64

The offspring given by an eosin vermilion equational daughter from table 63 when mated to a sable forked male.

No.	Regular offspring				Exceptions		
	f♀	+♀	$w^e vf$♂	$w^e v$♂	$w^e v$♀	sf♂	Percent
1044	51	43	48	48	6	2	4.

Two of the eosin vermilion exceptional daughters from table 64 gave regular offspring and reductional exceptions, but no equational exceptions (table 65).

TABLE 65

The offspring given by two eosin vermilion reductional exceptions from table 64 when mated to sable forked males.

No.	Regular offspring				Exceptions		
	f ♀	+ ♀	w^evf ♂	w^ev ♂	w^ev ♀	sf ♂	Percent
1143	85	90	73	69	8	9	5·7
1156	63	56	50	60	4	6	4·2
Total	148	146	123	129	12	15	

Five of the wild type regular daughters from table 64 tested by bar males gave no exceptions (table 66).

TABLE 66

The offspring given by five wild type regular daughters from table 64 when mated to bar males.

No.	Regular daughters	Regular sons														Exceptions	
		w^e v		s f		w^e s f		w^e v sf		w^ev f		w^e		s f		w^e s		w^evs		w^e f		+♀	B′♂
	B′	w^ev		sf		w^esf		v		w^evsf +		w^evf		s		w^e		vsf		w^es		vf		w^evs		f		w^ef		vs			
1154	40	12	13	9	6	—	2	—	6	—	—	1	—	2	2	—	1	—	—	—	—	—	—	—	—	—	—	—
1155	190	36	37	13	36	12	11	10	7	1	1	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1198	139	30	26	15	15	5	5	5	5	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1212	78	17	16	9	6	3	1	6	2	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1233	196	58	73	14	20	4	4	10	13	2	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	653	153	165	60	63	24	23	31	33	3	1	8	4	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—

In an experiment which is reported in the following section, a wild type XXY female which carried eosin in one X and whose other X was unmutated, produced an eosin exceptional daughter (table 77, culture 1221). Since there were no control characters nothing was done with the exception.

In a sister culture (number 1217, table 77) an XXY wild type female which carried eosin and vermilion in one X and neither in the other, produced two eosin and two vermilion equational daughters. Each of these four exceptions contained one crossover and one non-crossover chromosome, as the tests in table 67 show.

TABLE 67

The offspring given by two eosin and two vermilion equational daughters from table 77 when outcrossed to sable forked males.

A. Offspring from the two eosin daughters.

No.	Regular offspring			Exceptions		
	+ ♀	$w^e v$ ♂	w^e ♂	w^e ♀	sf ♂	Percent
1352	94	35	45	1	3	2.2
1403	106	66	51	6	2	3.5
Total	200	101	96	7	5	

B. Offspring from the two vermilion daughters.

No.	Regular offspring			Exceptions		
	+ ♀	$w^e v$ ♂	w^e ♂	w^e ♀	sf ♂	Percent
1408	206	104	92	4	7	2.7 ($w^e v$ ♀ 1)
1566	26	10	14	1	1	3.8
Total	200	114	106	5	8	

One of the four produced a further equational exception, which was an eosin vermilion daughter (culture 1408, table 67).

A large number of the wild type regular daughters from these four cultures of table 67 were mated to black males (table 68). One which carried eosin and vermilion in one X and sable and forked in the other, produced an eosin sable equational daughter (culture 1553).

TABLE 68

The offspring given by the regular wild type daughters from table 67 when outcrossed to black males.

A. Offspring from daughters from culture 1352.

No.	Females	Males	Equational exceptions
1527	29	51	—
1528	98	105	—
1529	137	149	—
1530	75	54	—
1531	101	112	—
1533	37	37	—
1535	132	114	—
1536	78	61	—
1537	100	93	—
Total	709	776	—

B. Offspring from culture 1403.

1547	107	149	—
1548	75	83	—
1551	96	97	—
Total	278	329	—

C. Offspring from culture 1408.

1552	69	62	—
1553	68	65	1 (w^{e}s ♀)
1578	174	141	—
1580	94	99	—
1581	145	154	—
1582	113	107	—
1584	117	104	—
1585	138	107	—
1586	126	122	—
Total	1044	961	—

This exception in culture 1553 was tested by black males (table 69).

TABLE 69

The offspring given by the eosin sable equational exceptional daughter from table 68 when tested by black males.

No.	Regular offspring			Exceptions		
	Black ♀	+ ♀	$w^e s$ ♂	$w^e s$ ♀	+ ♂	Percent
1669	5	5	5	1	—	6.3

The fact that half the daughters were black is proof that no error has occurred. Both of the X's were crossovers between eosin and vermilion and at least one was a crossover between sable and forked, that is, a double crossover. Whether the other X was also a double crossover or not is uncertain because the sons were too few in number (5) to show that the mother was not heterozygous for forked.

Another equational exception, the fifteenth case, was a yellow white daughter produced by a mother carrying yellow and white in one X and eosin and vermilion in the other (table 78, culture 1285). This female died by accident so that it is not known whether she was heterozygous for vermilion or not.

In culture 1424, table 34, an XX wild type female carrying eosin and vermilion in one X and sable and forked in the other produced by primary non-disjunction a wild type female and an eosin vermilion female. The eosin vermilion female was sterile, an unexplained circumstance. The wild type female died by accident.

In table 25, culture 1657, a wild type XXY female carrying yellow, eosin and vermilion in one X and no mutant genes in the other, gave a yellow eosin daughter which the test showed was heterozygous for vermilion (table 70).

TABLE 70

The offspring given by the yellow eosin equational daughter from table 25 when mated to bar males.

No.	Regular offspring			Exceptions		
	B′ ♀	$yw^e v$ ♂	yw^e ♂	yw^e ♀	B′ ♂	Percent
1727	141	60	47	13	11	8.8

In table 45, culture 1715, a wild type XXY female carrying eosin, vermilion and forked in one X and no mutant genes in the other, produced a vermilion daughter which was heterozygous for both eosin and forked. One X must therefore have been a double crossover and the other a non-crossover (table 71).

TABLE 71

The offspring given by the vermilion equational daughter from table 45 when mated to sable males.

No.	Regular offspring					Exceptions		
	+ ♀	w^evf ♂	v ♂	w^ev ♂	vf ♂	v ♀	s ♂	Percent
1736	91	20	31	19	21	2	4	3.2

There have been eighteen instances of equational exceptions. In thirteen of these cases, crossing over is known to have occurred, and this is *in every case where evidence upon this point was obtainable.* Again, of these thirteen cases which involved crossing over, *twelve were crossovers in only one chromosome and were non-crossovers in the other.* The thirteenth case was a double crossover in one chromosome and at least a single crossover in the other. Although fifteen of the instances occurred in XXY females, three occurred in females known to be simply XX, and it is thus evident that equational non-disjunction is not caused by the presence of a Y. The descendants of equational exceptions seem to have a greater tendency to produce further equational exceptions, though no basis for this tendency has been found.

As previously explained, all these exceptions are easily accounted for in the following way: XX synapsis took place; each X split so that a four strand stage occurred; crossing over took place between two only of these strands, one from each X; the reduction division separated the paternal X from the maternal X, each cell receiving a non-crossover and at the same time a crossover strand; at the next division these two strands ordinarily enter different cells, but by an occasional non-disjunction these two strands do not separate from each other at the equational division and consequently enter the same nucleus. In the case of an XX female the presence of the Y might favor the process by entering the other cell so that one cell receives two X chromosomes and the other two Y's. Equational non-disjunction thus enables us to examine at leisure the products of reduction.

It is impossible that a non-crossover and a crossover chromosome

come from a cell in which only two strands are present. Therefore the proof that these exceptions arise from XX (or XXY) oöcytes would at the same time prove that crossing over took place in this manner at a four strand stage.

If, however, the oöcyte contained three X chromosomes, synapsis and crossing over might occur between two of them and the equational split appear after the crossing over had been accomplished. At the reduction division the two synapsed X's (which would also be crossovers in a certain percent of cases) would disjoin and pass to separate poles, while the free X, which would always be a non-crossover, would go with one or the other according to chance. In those cases in which crossing over has occurred and a crossover and the free non-crossover X have remained in the egg after reduction, the equational division would make no quantitative change. These eggs fertilized by Y sperm would give exceptions having all the characteristics of those just described.

But none of these equational exceptions came from XXX mothers; for it is quite certain that XXX females do not live. It is possible, however, that a small group of XXX cells in an XX female might live if such cells were produced. Let us suppose that in a germ-tract division the two daughter chromosomes from one X were late in separating from each other, while the other X divided normally. Then both these X's might become caught in one cell as in other cases of primary non-disjunction. One cell from such a division would be X and the other X X′ X′ in composition, having two maternal (or paternal) X's and one of the contrary type. This X X′ X′ cell might give rise to a group of such cells in the germ-tract and these might cause the production of several equational exceptions in a single culture, as in culture 1217, table 77.

The twelve cases in which one of the X's was a non-crossover and the other a crossover chromosome are equally well explained on either the chiasmatype or the X X′ X′ view. The thirteenth case in which one X was a double crossover and the other X probably also a double crossover could be explained on either view as a case of primary non-disjunction at the second maturation division.

Yet if an equational exception should arise such that one of its X's were a double crossover and the other a single crossover between two of the same genes involved in the double crossover, then the X X′ X′ view could not be applied, for one strand cannot cross over at the same point with each of two other strands. Of the two exceptions which might

have answered this question, one did not give enough offspring to settle its nature and the other could have arisen by either method. The evidence in hand thus allows us no conclusion as to whether crossing over takes place at a two or four strand stage; but equational non-disjunction offers the possibility of answering this question definitely.[9]

SOMATIC NON-DISJUNCTION AND GYNANDROMORPHISM

If the same sort of primary non-disjunction which has been assumed to give rise to X X′ X′ cells in an XX female, should take place at a cleavage stage, gynandromorphs and mosaics would result. One might expect at an early cleavage division, particularly the first, a relatively large number of X—X X′ X′ divisions, for the greatly condensed chromosomes introduced by the sperm do not for some time attain the state or the appearance of those of the egg nucleus. If the paternal X of a female were slower than the maternal in preparing for division, it might lag upon the spindle so that both daughter X's would become included in the same cell. The portion of the fly which comes from the X cell should be male and should show the sex-linked characters of the mother. Such a process may be the explanation of the large number of lateral gynandromorphs of Drosophila. When the X—X X′ X′ division occurs at a later cleavage stage we may have mosaics, as for example, a red female with a patch of white facets in the eye.

HIGH NON-DISJUNCTION

The mean of the percentages of exceptions given by all the XXY cultures is 4.3 percent and the mode is at about 2.3 percent. Occasionally rather high percentages occur. For example, one of three regular white-eosin daughters from culture 800 gave nearly 14 percent of exceptions, while a sister gave 8 percent (table 73).

[9] The conclusive evidence that crossing over does take place at a four strand stage according to the chiasmatype hypothesis came Dec. 16, 1915, with the discovery of an equational female one of whose X chromosomes had undergone double crossing over while the other had undergone single crossing over at the same level (between the same two genes) at which the first crossover of the double had occurred. The mother of this exception was an XXY female of the constitution $\frac{v\ \ f}{\quad sg}$; the two recessive characters vermilion and forked were in one X, and the two recessive characters, sable and garnet, were in the other (for garnet see p. 151). The equational exception was of the constitution $\frac{v\ \ sg}{\quad\quad}$; the chromosomes represented by the space below the line (unmutated genes only) is a double crossover, and the chromosome represented above the line is a single crossover which had taken place between vermilion and sable, that is, in the same region in which the first single of the double had occurred.

TABLE 73

The offspring given by three white-eosin regular daughters from table 49 when outcrossed to wild males.

No.	Regular offspring		Exceptions		
	+ ♀	♂ ♂	w-w^e ♀	+ ♂	Percent
931	80	112	1	1	1.
932	77	74	11	13	13.7
933	113	112	11	8	7.8
Total	270	298	23	22	

From each of these cultures two exceptional daughters were tested. Each gave percentages somewhat higher than normal (table 74).

TABLE 74

The offspring given by four white-eosin exceptional daughters from table 73 when outcrossed to bar or wild males.

A. Two daughters from culture 932 mated to wild males.

No.	Regular offspring		Exceptions		Percent
	+ ♀	♂ ♂	w-w^e ♀	+ ♂	
1079	158	122	14	14	9.4
1080	157	159	6	10	4.8
Total	315	281	20	24	

B. Two daughters from culture 933 mated to bar males.

1039	129	123	14	6	7.4
1040	127	121	4	9	5.
Total	256	244	18	15	

Three exceptional daughters from culture 1039 (table 74) gave only low percentages of exceptions (table 75).

TABLE 75

The offspring given by three white-eosin exceptional daughters from table 74 when outcrossed to wild males.

No.	Regular offspring		Exceptions		
	+ ♀	♂♂	w-w^e ♀	+ ♂	Percent
1151	92	92	5	1	3.2
1152	83	95	5	2	3.8
1153	97	77	1	1	1.1
Total	272	264	11	4	

Two similar exceptional daughters from culture 1079 (table 74), gave high percentages of exceptions (table 76).

TABLE 76

The offspring given by two white-eosin exceptional daughters from table 74 when mated to bar males.

No.	Regular offspring		Exceptions		
	B′ ♀	♂♂	w-w^e ♀	B′ ♂	Percent
1184	110	80	11	9	10.0
1202	5	13	15	14	62.0
Total	115	93	26	23	

Up to this point none of the cultures have been so aberrant that they do not fall into the normal curve.

However, one of the cultures of table 76 produced *more exceptions than regular offspring, and this culture seems to belong to a different category of non-disjunction.*

Twelve of the regular daughters from culture 1079 were tested and one (culture 1164, table 77) likewise gave a remarkably high percentage of exceptions.

TABLE 77

The offspring given by the regular wild type daughters from table 74, culture 1079, when mated to bar males.

No.	Regular offspring					Exceptions		
	B′ ♀	w^{e}v ♂	+ ♂	w^e ♂	v ♂	+ ♀	B′ ♂	Percent
1182	89	29	25	11	11	4	4	4.6
1204	8	2	3	—	2	—	—	—
1205	26	12	3	2	3	3	4	13.2
1217	116	37	34	20	17	9	5	5.9 (w^e ♀ 2, v ♀ 2)
1222	6	5	3	2	1	—	—	—
1164	30	—	13	6	—	20	15	42.
1183	78	—	36	40	—	—	—	—
1203	91	—	38	42	—	—	—	—
1206	15	—	6	8	—	1	5	16.6
1221	97	—	47	32	—	7	6	6.9 (w^e ♀ 1)
1163	108	108 unclassified males				—	—	—
1185	85	110 unclassified males				—	—	—

Thirteen of the exceptional daughters from culture 1164, table 77, were outcrossed to bar males. The mean of the percentages of exceptional offspring was 5 percent, which is about normal, and there were no very high percentages (table 78).

TABLE 78

The offspring given by the wild type exceptional daughters from culture 1164, table 77, when mated to bar males.

No.	Regular offspring			Exceptions		
	B′ ♀	w^e ♂	+ ♂	+ ♀	B′ ♂	Percent
1238	148	52	53	3	3	2.3
1239	154	71	73	18	8	8.0
1240	188	90	84	8	7	4.0
1245	87	40	36	5	2	4.1
1246	146	83	59	3	3	2.0
1247	159	92	90	7	9	4.5
1248	100	47	52	8	6	6.6
1249	169	97	92	10	6	4.3
1250	154	85	85	6	7	3.9
1251	166	71	67	8	10	5.6
1252	152	68	77	11	9	6.3
1253	91	52	43	8	1	4.6
1289	182	88	84	18	11	7.6
Total	1896	936	895	113	82	5.0

Four of the regular bar daughters from culture 1164 were mated by white males. Only one produced exceptions, and the percentage was normal (table 79).

TABLE 79

The offspring given by four bar regular daughters from culture 1164, *table* 77, *when mated to white males.*

No.	Regular daughters				Regular sons				Exceptions	
	w-w^e	B′	w-w^{e}B′	+	w^e	B′	w^{e}B′	+	w ♂	Percent
1256	50	71	41	45	57	42	40	48	9	4.6
1257	21	19	11	10	26	16	10	10	—	—
1255	25	22	21	23	22	18	18	15	—	—
Total	46	41	32	33	48	34	28	25	—	—
1254	—	38	—	45	—	20	—	39	—	—

From the other unusually high culture (1202, table 76), four exceptional daughters were tested by mating to wild males. The percentages of exceptions were rather high (table 80).

TABLE 80

The offspring given by four white-eosin exceptional daughters from culture 1202, *table* 76, *when outcrossed to wild males.*

No.	Regular offspring		Exceptions		
	B′ ♀	Unclassified males	w-w^e ♀	+ ♂	Percent
1287	135	111	11	8	7.3
1288	135	137	3	13	5.6
1302	114	131	13	8	7.9
1336	130	113	8	13	8.
Total	514	492	35	42	7.1

Four other exceptional daughters tested by bar brothers gave normal percentages of exceptions (table 81).

These tests of the regular and exceptional daughters from the two unusually high cultures seem to show that the tendency to produce very high percentages is not due to anything which has happened to the X chromosome. If some sort of mutation in one or both of the X's was

TABLE 81

The offspring given by four white-eosin exceptional daughters from culture 1202, table 76, when mated to bar brothers.

No.	Regular offspring		Exceptions		
	B′ ♀	Unclassified males	w-w^e ♀	B′ ♂	Percent
1258	124	105	9	2	4.6
1285	174	176	11	8	4.9(yw ♀ 1)
1286	134	114	4	9	5.
1303	164	166	11	9	5.7
Total	596	561	34	28	5.1

responsible for this high non-disjunction, then all exceptional daughters, which receive both the X's of the mother, should also give high non-disjunction, but this was not the case.

Since secondary non-disjunction is caused by the presence of the Y chromosome, it seemed possible that the change in production of exceptions might be due to a change in the Y chromosome, that is, to a Y mutation. A high-producing female was assumed to be XXY′. Y′ would descend to all of the exceptional sons of such a female, and also to half of the regular daughters. All the exceptional sons of an XXY′ mother should be XY′ (see figure 6, p. 13). Since the effect of Y′ can only be detected in an XXY′ female, three of the exceptional bar sons (XY′) from culture 1202, table 76, were outcrossed to XXY exceptional daughters from table 44.

The XXY females to which the XY′ male is crossed should of course produce only the normal percentage of exceptions, as in fact they did (see cultures 1306, 1333, and 1304, table 45). All the exceptional daughters from these cultures should however be XXY′ and should give high non-disjunction. Two such daughters were tested by bar males and one gave very high non-disjunction (see culture 1417, table 45). The other culture, 1418, gave a rather low normal percentage.

The results are contradictory, but the partial success of the test encouraged further tests of the same nature. An attempt was made to secure a stock in which every Y, whether in females or in males, should be Y′. If culture 1417 was high because of the presence of Y′, then, as has been shown, the exceptional sons of this culture should also be XY′.

Since the father of culture 1417 was an ordinary bar male, all the exceptional daughters should be XXY and not XXY′. Matings between

these exceptional XXY daughters and XY′ sons should give normal percentages of exceptions, as was the case (see cultures 1612, 1613, 1618 and 1624, table 45).

The exceptional daughters from this last cross should be XXY′ and should give high non-disjunction. Three of them were mated to their *fathers* (which had been saved for this purpose), and since their Y's came from their fathers each of these cultures should give a line in which every Y is mutant. Also the percentages should be high from the XXY′ composition of the mother. Three such daughter by father matings were made and each gave a percentage above normal, but not unusually high (cultures 1703, 1714, 1715).

Evidently then, if there is a high line, the mode is in the neighborhood of 8 or 9 percent, with occasional very high cases. All the data secured in these tests will bear such an interpretation, but it cannot be considered proved until extensive tests of the percentages of exceptions given by the pure Y′ lines have been made.[10]

KEEPING STOCK

The method usually followed in keeping stock of non-disjunction is to breed from the exceptional females of any culture. These females have an extra Y chromosome and give exceptional daughters like themselves, and exceptional sons like whatever male was used in the outcross. In carrying on the stock in this fashion it is of course necessary that the females be virgin, and this involves the inconvenience of watching the stock very closely, as the exceptional females are rare at best. A method which does not require such continuous attention is to mate an exceptional female, say eosin-eyed, to several of her brothers having eosin eyes. This will give an eosin stock which can be transferred from generation to generation. But in the use of such a stock, special precautions have to be taken, for not all the females will give exceptions when outcrossed. The initial eosin female had an extra Y, so that half of her sons and daughters will have one also. Moreover half of the eosin brothers to which such females are mated have an extra Y, so that an additional supply of Y's come from them. Only about three-eighths of all the flies of a freshly made stock will escape having an extra Y, while about one-eighth will have two extra Y's. In further generations of this stock the percentage of flies which have extra chromosomes may decrease. In carrying on a stock by a *few* parent flies in each generation, any mating which chances to

[10] Recently such tests have been made and they show that the mean of the high line is about 20 percent of exceptions.

have less than the average richness of Y's can not recover in the next generation, so that any particular poor selection will be a permanent injury to the stock.

In using such a stock one must make several P_1 matings in order to insure getting one in which the female carries an extra Y. As soon as such pairs have produced offspring one can select a culture which has given exceptions and discard the others. Of twenty-six such matings made from fresh stocks, only six failed to give exceptions (table 82).

TABLE 82

The three kinds of results given by eosin females of fresh stocks of non-disjunction when tested by wild males.

No.	Regular offspring		Exceptions		Percent of exceptions
	+ ♀	w^e ♂	w^e ♀	+ ♂	
62n	130	122	2	1	1.2
63n	69	60	6	5	7.9
64n	70	84	1	—	.6
66n	43	24	3	4	9.5
68n	64	64	4	5	6.6
69n	94	91	7	10	8.4
70n	72	52	6	16	15.1
71n	65	48	4	3	5.8
88n	59	62	1	4	4.
89n	45	59	—	2	1.9
90n	50	52	1	2	2.9
91n	24	27	—	1	1.9
92n	83	76	3	2	3.
93n	81	64	6	—	4.
95n	50	40	1	2	3.2
96n	37	42	1	—	1.3
140n	82	80	3	4	4.1
141n	33	30	2	—	3.1
142n	53	50	1	—	1.
145n	22	32	—	1	1.8
Total	1226	1159	52	62	—
61n	49	56	—	—	—
65n	121	119	—	—	—
67n	75	79	—	—	—
94n	79	77	—	—	—
97n	44	35	—	—	—
128n	110	90	—	—	—
Total	478	456	—	—	—

Tests made with a stock which had been maintained by transference in this manner through five months showed little deterioration, since four out of six females tested gave exceptions (table 83).

TABLE 83

The offspring given by six eosin females, from a stock transferred through five months, mated to bar males.

No.	Regular offspring		Exceptions		
	B′ ♀	w^e ♂	w^e ♀	B′ ♂	Percent
119n	50	48	—	1	1.
121n	54	54	—	1	1.
122n	68	71	7	8	9.7
124n	45	63	1	1	1.8
Total	217	236	8	11	—
120n	82	82	—	—	—
123n	41	48	—	—	—
Total	123	130	—	—	—

However, from another stock which had been kept about five months only four females out of the twenty-three tested gave exceptions (table 84).

TABLE 84

The offspring given by eosin females, from a stock transferred through eight generations, when mated to vermilion males.

No.	Regular offspring		Exceptions		Percent
	+ ♀	w^e ♂	w^e ♀	v ♂	
295	35	40	—	1	1.3
319	54	38	—	1	1.1
321	17	19	1	—	2.3
322	10	14	—	1	4.1
Total	116	111	1	3	—
296	55	54	—	—	—
297	38	14	—	—	—
298	87	61	—	—	—
299	35	37	—	—	—
307	35	21	—	—	—
308	64	31	—	—	—
309	53	53	—	—	—
310	35	27	—	—	—
311	31	29	—	—	—
312	31	26	—	—	—
313	29	17	—	—	—
314	11	10	—	—	—
315	29	23	—	—	—
316	31	23	—	—	—
317	52	21	—	—	—
318	35	24	—	—	—
320	43	28	—	—	—
323	61	50	—	—	—
324	52	47	—	—	—
330	34	30	—	—	—
331	35	31	—	—	—
332	38	33	—	—	—
Total	914	690	—	—	—

It will be noticed that not more than one exception appeared in any of the cultures of this table. It is very possible that some of these may be primary exceptions, in view of the frequency with which primary non-disjunction must be supposed to occur. Moreover, all three of the regular daughters of culture 321 which were tested failed to give exceptions, as would be the case if the exception in 321 were primary (table 85).

TABLE 85

The offspring given by three wild type regular daughters from culture 321, table 84, when mated to bar males.

No.	Regular offspring		Exceptions	
	B′ ♀	Unclassified males	+ ♀	B′ ♂
389	105	94	—	—
390	102	123	—	—
391	78	66	—	—
Total	285	283	—	—

For the most part there has been no need for special stock of non-disjunction, since the experiments have continually furnished material for fresh work. Those few cultures which have had little purpose aside from keeping stock are summarized in table 86. The pedigree gives the relation of each culture to the whole fabric. Culture 339 was intended as part of an experiment with certain eye-colors, but since it gave secondary exceptions of a convenient kind a new line of non-disjunction was started and the old line was terminated.

TABLE 86

Miscellaneous cultures.

Parents	No.	Regular daughters	Regular sons	Exceptions	Percent of exceptions
w^e ♀ × w ♂		w-w^e	w^e	w^e ♀ w ♂	
	43n	82	88	8 12	10.5
w^e ♀ × wB′ ♂		w-w^eB′	w^e	w^e ♀ wB′ ♂	
	42n	66	88	5 2	4.4
+ ♀ × wB′ ♂		w-w^eB′ B′	w^e +	+ ♀ wB′ ♂	
	58n	18 25	25 32	1 2	2.9
	60n	19 39	32 26	2 2	3.3
		37 64	57 58	3 4	
w^e ♀ × B′ ♂		B′	w^e	w^e ♀ B′ ♂	
	152n	40	24	2 2	5.9
	153n	37	38	2 5	8.5
		77	62	4 7	
v ♀ × + ♂		+	v	v ♀ + ♂	
	339	77	73	10 10	11.8
v ♀ × B′ ♂		B′	v	v ♀ B′ ♂	
	419	190	160	3 4	2.0
	420	164	169	3 1	1.2
	421	189	159	2 —	0.6
	429	205	142	2 3	1.4
	430	106	97	2 —	1.0
		854	727	12 8	
+ ♀ × wB′ ♂		w-w^eB′ B′	w^e v w^ev +	+ ♀ wB′ ♂	
	649	60 63	46 38 20 16	3 2	2.0

MUTATIONS

The cultures reported in this paper have given rise to over a dozen mutations, and these may be briefly described.

Cream a (July 15, 1915). It was noticed that some of the eosin males of culture 43n (table 86) were considerably lighter than normal and were a pale yellow or "cream" color. A "cream" male was outcrossed to a wild female and gave wild type sons and daughters as expected if the cause of the dilution were recessive. An F_1 pair produced

a surprising F_2 result, which differed from the result of the simple F_2 from an eosin male by wild female only in that a quarter of the flies which were eosin were diluted to cream. None of the not-eosin flies showed a trace of dilution. The character "cream" is then a double recessive, the product of the action of a recessive autosomal gene ("cream," by extension) added to the effect of the sex-linked gene eosin. However, the single recessive, cream, is indistinguishable from red, that is, by itself it produces no visible effect. Cream *a* was the first of a class of mutations which are specific diluters of eosin, that is, which require the presence of eosin before they can develop any visible effect. These specific modifiers are analogous to the modifiers which extend or restrict the area of the white pigment of the hooded rat, and which produce no visible effect unless the white-producing gene is present as a base.

Dark and *whiting* (Sept. 23, 1913 and Nov. 21, 1913). It was noticed that in the sister cultures 100n and 101n (table 16), there were present eosin males which were abnormally dark in color. In the next generation more dark males appeared, and some of the white-eosin females were practically as dark as pure eosin. In subsequent generations this dark modification appeared in a manner and in such proportions as to suggest that it was a case converse to cream *a*, that is, that there was present a recessive gene which is a specific darkener of eosin, and which does not modify the color of those flies which are not eosin.

One pair of "dark" eosin flies gave about a quarter of the offspring pure white in eye-color (Nov. 21, 1913). This was an astonishing result, since the sex-linked white could not appear among the daughters of such a culture except by the rare equational non-disjunction. Other features of the cross were likewise impossible on the assumption that the colorless eye was the sex-linked white. A series of tests showed that this white color was in fact not the sex-linked white which had been running through the experiments up to this point, but was a new and complex color due to a specific diluter of eosin, that the white was a double recessive, eosin "whiting." It is remarkable that a gene which has so profound an effect upon eosin (depriving it of all trace of color in both males and females) should by itself produce no visible effect; the stock of whiting is indistinguishable from a wild stock, and the eosin whiting is indistinguishable from white. How curiously specific is the dilution effect of whiting, is illustrated by the fact that whiting does not affect cherry, an allelomorph of eosin so similar to eosin that cherry and eosin females are practically indistinguishable.

Cream b (March 10, 1914). In culture 82 (table 41) a single eosin male somewhat lighter in color than the others was observed. This dilution was found to be due to an autosomal diluter like cream *a* though not as marked in its effects.

Three other specific diluters of eosin have come up in my experiments. A remarkably close imitation of such a multiple factor case as that of CASTLE's hooded rats could be concocted with the chief gene eosin for reduced color, and these six diluters which by themselves produce no effect, but which carry the color of eosin through every dilution stage from the dark yellowish pink of the eosin female to a pure white.

Lethal 4 (March 13, 1914). The same culture, 82 (table 41), which gave rise to cream *b* gave only half as many sons as daughters. The cause of this absence of sons was found to be a sex-linked lethal gene whose locus in the X chromosome is at approximately 49.

Lethal 6 (April 9, 1914). The cream *b* male of culture 82 (table 41) was outcrossed to a wild female, and a pair of F_1 flies gave only half as many sons as daughters. These sons were all eosin, so that the death of the wild type sons must have been due to a lethal (lethal *6*) which was carried by the X from the wild grandmother. This lethal is interesting since it has been found to lie to the left of yellow at a position —.04. This is the closest "partial" linkage recorded, there being only one crossover between lethal *6* and yellow in 2500 flies.

Spoon (December 12, 1914). In culture 898 (table 37 (*B*)) a male was found which had a thin-textured wing, curved like the bowl of a spoon and having an extra cross vein. This mutation proved to be a sex-linked recessive which lies to the left of eosin and close to yellow. However, only rarely do spoon flies hatch, so that the character has to be handled like a lethal, with the exception that the occasional spoon male can be used in mating—an advantage lacking in the case of total lethals. This mutation is a sort of connecting link between those mutations, such as rudimentary, which are poorly viable under adverse conditions, and total lethals which never hatch under any condition. One class of lethals can be regarded as an extension of this inviability effect to the point at which the mutant individuals can no longer hatch.

Lethal 7 (January 1, 1915). Another category of lethals is illustrated by lethal *7* which appeared in culture 1072 (table 61). This lethal, which also lies to the left of eosin, causes the death of the individual at the mature larval stage. Those larvae which are about to die can be separated from those which are to live, because the morescent larvae,

when half mature, leave the food and wander about on the surface of the culture bottle. Furthermore, while these larvae are still young one or more intense black specks appear in their body-cavities. As the larvae become older this character, which is absent from normal flies, becomes more conspicuous, so that one can easily pick out those cultures in which the mother was heterozygous for lethal *7* by the numbers of black-spotted larvae wandering about or dead upon the walls of the bottle. Lethal *7* is then a larval character of such virulence as to cause a change in the instincts of the individual and finally to cause its death.

Deficiency (September 25, 1914). Still another and the most significant category of lethals was found in the appearance in culture 546 (table 9) of a white-eosin daughter from a mother heterozygous for eosin and a father which was white but also bar. *This daughter received the white from the father but did not receive his bar.* Since both white and bar are carried by the X chromosome, either bar remutated to normal or something happened to the region of the X in which bar was situated. Tests showed that the latter explanation was correct, that a small section of the X in the bar region had become genetically non-existent! This X, at the same time that it lost the gene for bar, lost the normal allelomorph for forked, which is about half a unit from bar. Females having one normal X and one deficient X, outcrossed to forked males, produced daughters half of which were forked, and this in spite of the fact that forked is a strict recessive. These forked daughters have forked in the paternal X and in the maternal (the deficient X) no allelomorph of forked whatever. This deficient region is known to extend from forked to bar and to fail to extend to rudimentary (rudimentary is 1.4 units to the left of forked) on the one hand and to fused on the other (fused is 2.3 units beyond bar). The existence of normal genes and their allelomorphs between the deficient region and the end of the X proves that the end is not gone but that the change is an internal one which affects a specific region of the hereditary material. A female having one deficient X shows no crossing over in the region between forked and bar. The section of the X chromosome from forked to bar exhibits properties like those of the Y chromosome; perhaps the lack of genetic material in the Y has been brought about through the same process which occurred in the X of the white bar male.

A female having one deficient X gives only half as many sons as daughters, that is, a male is unable to live unless he has a whole X.

It should be noticed that this first case in which a true "loss" of

genetic material is known to occur is unaccompanied by any visible mutative change; the female having a section of the essential genetic material gone from one X is entirely normal in appearance. This is of great significance since the loss involves the region in which the dominant bar is situated. If the dominance of bar were due to the loss of an inhibitor then this female should have been bar, which she was not. The expedient of explaining a dominant as the loss of an inhibitor is useless in this case and of extremely questionable value in most other cases.

Of considerable theoretical importance is the fact that an individual having one deficient chromosome is a *female,* normal in appearance and function. Therefore it is not essential for the production of a female that two whole X's be present, that is, sex production is a function of some particular part of the X rather than of the X as a whole; further, the sex differentiators are situated in some region of the chromosome *other than the region from forked to bar.* It may be long before we know in what region the sex differentiators are, but meanwhile it is some satisfaction to know where they are not.

Short (July 20, 1914). In culture 310 (table 84) all the males that were eosin had wings of about half or two-thirds normal length and of conspicuous broadness. This mutation proved to be sex-linked and to be located between yellow and white.

Bifid (July, 1914). In culture 323 (table 84) the sex-linked mutation bifid reappeared.

Rudimentary (July, 1914). In culture 330 (table 84) the sex-linked mutation rudimentary reappeared. Both bifid and rudimentary were checked by the presence of eosin, and by the fact that they occurred at Cold Spring Harbor where there were no stocks of these mutations.

Confluent (September 23, 1914). In culture 550 (table 9) appeared a single male with the veins of the wing thickened and knotted and confluent with the marginal vein. This mutation was a second-chromosome dominant, which was probably lethal when homozygous (yellow mouse case). The interesting feature of this mutation was that C. W. METZ and B. S. METZ (1915) found in another species of Drosophila a mutation which corresponded with this mutation in all points, both as to its somatic appearance and genetic behavior.

Garnet (February 19, 1915). In culture 1347 (table 34) one of the sable sons had an eye-color which was practically indistinguishable from

purple (purple is a second-chromosome mutation). The new color "garnet" proved to be sex-linked and to be located at about 44.4.

III

OTHER EXCEPTIONS TO SEX-LINKED INHERITANCE, AND VARIATIONS IN THE DISTRIBUTION OF CHROMOSOMES

In several other forms exceptions occur which seem to be of the same type as those found in Drosophila,—namely, matroclinous daughters and patroclinous sons. Cytological work has shown that in many forms the chromosomes are subject to variations in distribution and constitution. While it is not probable that all these variations can be fully explained by non-disjunction, yet it may be profitable to point out how far they may be accounted for on this theory.

Abraxas

One of the most extensive cases to which the explanation of non-disjunction seems to apply is that of the exceptions to sex-linked inheritance which DONCASTER has found in Abraxas. The wild form of Abraxas is *grossulariata*. A rare variety, *lacticolor*, characterized by smaller and paler markings, behaves as a recessive sex-linked character, as is shown by the cross of *lacticolor* male by *grossulariata* female, which gives *lacticolor* daughters and *grossulariata* sons. This "criss-cross" inheritance, which is characteristic of sex-linkage, is here of a type which is the converse of that found in Drosophila, and is explained by assuming that in Abraxas the female is heterozygous as regards the sex-differentiator and sex-linked characters. For convenience in referring to cases of the Abraxas type we may call the sex chromosome present in the homozygous sex, which in this sense corresponds to the X of Drosophila, a Z chromosome, while the chromosome corresponding in this sense to the Y of Drosophila may be called a W chromosome. The formulae are then:

	♂ = ZZ		XX = ♀
Abraxas type		corresponding to	Drosophila type.
	♀ = ZW		XY = ♂

DONCASTER found in Abraxas that both the female and the male have 56 chromosomes.

Normally in Abraxas the sex-ratio is approximately 1♀ : 1♂, but in one exceptional line the equality of sexes is not the rule. In this strain

DONCASTER (1913, 1914 a, b) found many females which gave only daughters and not a single son. Other females of this line gave many daughters but also a few sons, while still others gave practically a normal 1 : 1 ratio.

When DONCASTER examined this line cytologically, he found that although the males were normal, with 56 chromosomes, the females were aberrant and had only 55 chromosomes. Even those females which gave 1 : 1 ratios had only 55 chromosomes. In the maturation of the eggs of such a 55 chromosome female, the odd chromosome went to one pole, so that one polar plate had 27 and the other 28 chromosomes. DONCASTER found further that the odd chromosome went most often to the polar body, leaving a majority of the eggs after maturation with 27 chromosomes.[11] After fertilization, the many eggs which eliminate the odd chromosome become individuals with 55 chromosomes, that is, females, while the few eggs which retain it become 56-chromosome individuals, that is, males. The preponderance of females in the strain is thus explained. Such females, having only 55 chromosomes, would be of the type ZO. In females of normal strains there is a W chromosome present, but since this W chromosome may be absent without effect upon the sex of the individual, it must be regarded as functionless in determining sex, and in this sense corresponds to the Y of Drosophila. This evidence proves that there is present in Abraxas that cytological basis which the evidence from sex-linkage demands, namely, a condition the converse of that known in other groups of insects.

The loss of a chromosome from this strain with 55 chromosomes instead of 56 can be readily accounted for by primary non-disjunction. Suppose that in the male the two Z chromosomes should both pass at the reduction division into one cell, leaving the other cell with no Z. The sperm produced from the latter cell, fertilizing a Z egg, would give rise to a ZO female. The same result could be reached if both Z and W should pass out into the polar body of the egg and such an egg be fertilized by ordinary Z sperm, thereby producing a ZO female. The strain once established would continue automatically to give ZO females with 55 chromosomes and ZZ males with 56 chromosomes.

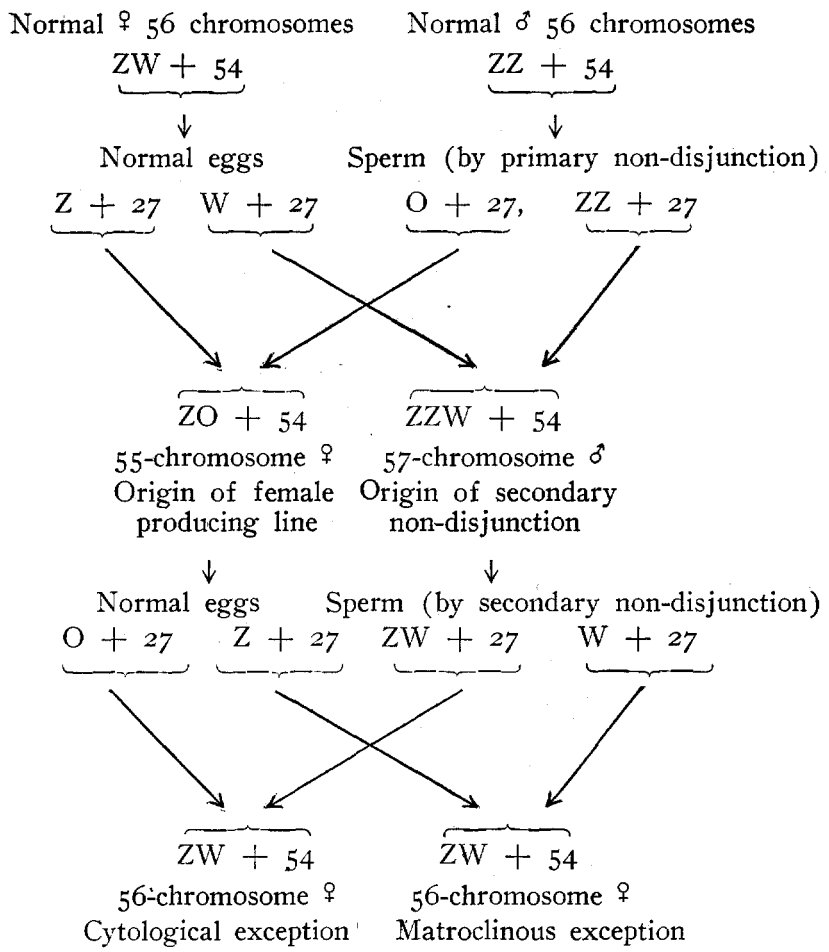
If non-disjunction is the explanation of the origin of the 55-chromosome female line, then we may expect to find other evidences of non-

[11] Recently DONCASTER has published a short note (Nature, June 10, 1915) in which he states that a more extensive examination of the reduction divisions has failed to show that the 27 chromosome eggs are more numerous than the 28. This contradiction in the evidence detracts much from the value of the case as it now stands.

disjunction in Abraxas. At various times DONCASTER has found genetic exceptions of the same character as are found in Drosophila, and in the case of some of his exceptions there can be small chance of error.

There is also cytological evidence derived from the examination of a certain female that occurred in one of the same broods that gave exceptions to sex-linked inheritance. This female had 56 chromosomes although she was from a strain of which the females normally have only 55 chromosomes. The additional chromosome in this female can be accounted for on the supposition that her father had a supernumerary W chromosome. Such a supernumerary could arise in a male through the fertilization of a W egg by a sperm which receives two Z chromosomes by primary non-disjunction. Such a ZZW male would produce some sperm having both Z and W. A ZW sperm fertilizing an egg without a Z would produce a ZW female with 56 chromosomes, such as DONCASTER found. In the same brood in which DONCASTER found this 56-chromosome female, he found two matroclinous daughters. These also can be readily accounted for in the following way. In the spermatogenesis of the ZZW father there would be formed some sperm having only W (corresponding to the Y eggs of Drosophila.) This W sperm fertilizing a Z egg of the ZO mother would give a ZW daughter which would have 56 chromosomes. But in this case the daughter would have received her Z chromosome from her mother, so that she should show the sex-linked character of the mother, that is, she should be a matroclinous exception like the two DONCASTER found in the brood.

That non-disjunction occurs in the wild Abraxas is indicated by the discovery of a wild female with 55 chromosomes. This 55-chromosome female with the formula ZO is the converse of the ZZW male required in the explanation above. The following diagram illustrates the probable origin of the ZO female line and of the ZZW males, and also of the exceptions observed.



If non-disjunction is the correct explanation of the case just reviewed, then we may hope to obtain females and males with 57 chromosomes. When exceptions to sex-linked inheritance occur, then half the sons and daughters in the brood should have a supernumerary, if the exceptions are due to secondary non-disjunction. If the exceptions are due to primary non-disjunction,—a much rarer phenomenon,—then only an occasional individual with the supernumerary should be found. The supernumerary would be only half as common in a cross in which the mother was herself without a W. The cross most favorable for observing exceptions and for obtaining supernumeraries would be that of a *lacticolor* male by a wild *grossulariata* female. If an exceptional son should appear in this cross, he should give some exceptional sons like

himself and some matroclinous daughters, since he would be of the composition ZZW. Furthermore, half of his sons and daughters should have 57 chromosomes and half 56.

The explanation which DONCASTER has suggested for some of these exceptions depends upon the assumption that the Z chromosome has divided into two chromosomes, one of which bears the locus for *lacticolor* and the other of which bears the sex-differentiator. As far as the evidence enables us to decide, this hypothesis explains some features of the case as well as does non-disjunction. The notable exception which it does not seem to explain is the production of the 55-chromosome line.

Paratettix

In Paratettix, NABOURS (1914) has reported a case of genetic exception for which two alternative explanations have been offered (DEXTER 1914). NABOURS collected nine distinct true-breeding wild forms, of which eight form a system of multiple allelomorphs or a system of practically complete linkage, while the form *melanothorax* (G) is independent of the others in inheritance. In "The mechanism of Mendelian heredity" reasons have been given for considering multiple allelomorphism the more probable explanation. This material is exceptionally favorable in one respect—that the color-patterns studied are superimposed in the hybrids so that the zygotic constitution of any individual can be determined by simple inspection.

In one of NABOURS's experiments, a BI female mated to a CE male, gave, as expected, equal numbers of the four classes BC, BE, CI, and EI, but gave in addition an exceptional individual of the constitution BIE.

If BI and E are members of a multiple allelomorph system, which is the most probable case, then in the egg which gave rise to this BIE individual, non-disjunction must have occurred whereby the B and I bearing chromosomes both remained in the egg. This BI egg fertilized by an E sperm from the CE father, would give the BIE individual. Such an individual should have three homologous chromosomes, and a total of one more than the number characteristic of the species. Genetically, also, the results should be very easy to test, for secondary non-disjunction should occur, wherefore half the offspring should be further triple forms of various constitutions. B and I should freely separate, for it is improbable that there would be preferential synapsis between these chromosomes which are not dissimilar, as they are in Drosophila in the case of XXY and XYY. On the other hand, if the case is one of linkage,

the BIE individual is explained as a crossover, and it should have no extra chromosome. In subsequent generations B and I should not freely separate but should remain coupled as strongly as they were repulsed before. It is to be hoped that another triple-charactered individual will be found, for genetic and cytological tests of it would be of exceptional interest. The study of non-disjunction of *autosomes* is extremely difficult in ordinary forms where the constitution of each individual must be found by laborious breeding tests, but in case the three chromosomes of the non-disjunctional individual each contain a different allelomorph of a multiple allelomorph series, and this triple form is distinguishable, then such a study should be easy.

Oenothera lata and Oe. semilata

A case of autosomal non-disjunction which has been thoroughly studied is that of *Oenothera lata* and *Oe. semilata* (LUTZ 1912, GATES and THOMAS 1914). The cytological and breeding work have shown that these forms are due to the possession of an extra chromosome, that is, one of the autosomes is triploid. *Lata* and *semilata* occasionally arise directly from *Oe. Lamarckiana,* and the explanation is found in the fact that occasionally in the gametogenesis of *Lamarckiana* a pair of synapsed chromosomes fail to disjoin and pass to the same pole (primary non-disjunction). The fact that the possession of three autosomes of one kind makes the individual visibly different from *Lamarckiana* is of great convenience in studying non-disjunction, for the offspring which possess a triploid chromosome do not have to be tested, as in the case of Drosophila, but can be readily distinguished as *lata* or *semilata.* Secondary non-disjunction occurs in the *lata;* at reduction the free chromosome passes to one pole or the other so that half the gametes are *aa* and half are *a.* This is not strictly true, for very often the free autosome lags upon the spindle and so fails to be included in either nucleus. A *lata* individual thus produces less than the expected percent of *aa* gametes. This is strictly paralleled by the result of crossing *lata* (*aaa*) to *Lamarckiana* (*aa*), for where this cross is expected to give half *lata* and half *Lamarckiana,* it rarely gives as many as half the plants *lata.*

Pigeons

Exceptions to the inheritance of the blond and the dark types of pigeons have been explained as partial sex-linkage (BRIDGES 1913 a), but non-disjunction offers an alternative explanation which seems more plausible.

Canaries

Exceptions to sex-linkage in the inheritance of pink versus black eye-colors have been reported (DURHAM and MARRYATT 1908). These exceptions are explainable by non-disjunction or by partial sex-linkage.

Fowls

BATESON and PUNNETT (1911) reported several exceptions in the inheritance of pigmentation of the silky fowl, which may be due to non-disjunction.

Aglia tau and A. lugens

STURTEVANT (1912) interpreted as partial sex-linkage the ratios observed by STANDFUSS (1896) in breeding *Aglia tau* and its variety *lugens*. This explanation seems to fit the data empirically and it is difficult to explain the results as due to non-disjunction. More recent data by STANDFUSS (1910) throw some doubt on this case. Similar results have been obtained occasionally in Drosophila, but have never been capable of repetition and are probably due to irregularities of viability caused by unfavorable culture-conditions. The hypothesis of partial sex-linkage has met a serious difficulty in the fact that in Drosophila and in the silk worm moth there is no crossing over in the heterozygous sex.

Cats

Apparent exceptions to sex-linkage in cats have been explained satisfactorily by WHITING (1915) without recourse to partial sex-linkage or to non-disjunction. WHITING offers evidence which shows that there are extension and restriction factors for yellow and black so that the apparent exceptions are simply modified classes which are expected.

Metapodius

WILSON (1907, 1909, 1910) found that in Metapodius the number of Y chromosomes varied from zero to six, and he offered the explanation that the origin of the variation was in an aberrant reduction division in which both sex chromosomes entered the same gamete. WILSON actually observed three reduction divisions in which X followed Y to one pole. This constitutes primary non-disjunction. The subsequent piling up of Y's was due to secondary non-disjunction. WILSON (1910) explained the mmm individual of Metapodius in the same fashion, by assuming the formation of an mm gamete.

Banasa

In *Banasa calva* WILSON (1907) found that the Y chromosome may be present or absent.

Diabrotica

STEVENS (1908 b, 1912 a) described in *Diabrotica soror* and *D. vittata* a variation in the number of Y's from one, the normal number, to five supernumeraries, or six Y's in all.

Ceuthophilus

A like variation was described by STEVENS (1912 b) in Ceuthophilus, where the number of supernumerary Y's was three.

These cases in Metapodius, Banasa, Diabrotica, and Ceuthophilus are cytologically of the same kind as that in Drosophila.

Ascaris

Miss BORING (1909) found in Ascaris a small extra chromosome which was interpreted as the X, which ordinarily is attached to the end of an autosome. S. FROLOWA (1912) found cases in Ascaris in which two of these X's seemed to be attached to the same autosome. These two X's would be carried into the same gamete and should give results comparable to non-disjunction.

IV

SUMMARY AND CONCLUSIONS

Evidence has been presented which proves that the occasional (1 in 1700) matroclinous daughter or patroclinous son produced by females known to be XX in composition is due to primary non-disjunction, that is, the X's fail to disjoin and are both included in the egg or both extruded to the polar cell.

The fertilization of the zero egg by an X sperm of a normal male results in a patroclinous XO son. He is entirely unaltered in somatic appearance, both as to sex-linked characters and as to sexual characters, but he is absolutely sterile. This difference between XO and XY males proves that the Y has some normal function in Drosophila.

The fertilization of an XX egg by a Y sperm of a normal male gives rise to a matroclinous daughter of the constitution XXY. The consti-

tution of a matroclinous female as XXY has been proved by direct cytological examination and by conclusive genetic tests.

Matroclinous females always produce further exceptions which we may call secondary, to the extent of 4.3 percent. The cause of this production is the fact that the presence of the extra Y forces both X's to enter the same cell in a certain percent of reductions.

In an XXY female the sex chromosomes do not synapse as a triad, but two synapse, leaving the third unsynapsed. Synapses are not at random, but are highly preferential; in 16.5 percent of cases Y synapses with one or the other X (heterosynapsis) and the remaining X is unsynapsed; in 83.5 percent of cases synapsis is between X and X (homosynapsis) and the Y is unsynapsed.

At the reduction division the two synapsed chromosomes disjoin and pass to opposite poles. The free chromosome goes with one or the other at random.

Reductions are not preferential; the polar spindle delivers two chromosomes to the polar cell as often as to the egg.

After XX synapsis the amount of crossing over is slightly increased (13.5 percent) in some manner by the presence of the extra Y.

After XY synapsis there is no crossing over—either between the X and Y or between the synapsed X and the free X.

After XY synapses the eggs are XX and Y, and X and XY. These four classes of eggs are in equal numbers and are non-crossovers.

The XX eggs by Y sperm give matroclinous daughters which are exact reproductions of the mother in all respects.

The Y eggs by X sperm give patroclinous sons which can give non-disjunctional effects neither in F_1 nor in F_2.

The X and XY eggs from XY synapses are indistinguishable from the non-crossover classes of the X and XY eggs which are from XX synapses. As a result the linkage values must be corrected.

The XY egg by X sperm gives an XXY regular daughter which nevertheless gives 4.3 percent of secondary exceptions by virtue of the extra Y.

YO, YY, and XXX zygotes are unable to live.

The XY egg by Y sperm gives XYY males. These males produce no exceptions in F_1 but produce XY sperm which, fertilizing X eggs, give rise to XXY daughters, and these produce secondary exceptions.

Synapses in an XYY male are probably at random.

The source of chromosomes, whether maternal or paternal, is without effect upon their subsequent behavior at synapsis and reduction.

The predominant type of non-disjunction has been shown to be pre-

ceded by XY synapsis and to take place at the reduction division. A rare type of non-disjunction takes place at an equational division. Equational non-disjunction is apparently always preceded by XX synapsis and crossing over. Equational non-disjunction offers the possibility of determining whether crossing over in Drosophila takes place by the chiasmatype (four strand stage) method or at a two strand stage.

Somatic non-disjunction explains the occurrence of gynandromorphs and mosaics in Drosophila.

Unusually high percentages of exceptions occur and are irregularly inherited. It is suggested that the cause of the high non-disjunction is a mutation in the Y chromosome.

The occurrence of non-disjunction in Abraxas is shown both by cytological and by breeding tests. Various other forms, namely, canaries, fowls, pigeons, and the moth, *Aglia tau,* show exceptions to sex-linkage which may be explained as due to non-disjunction.

In *Oenothera lata,* autosomal non-disjunction has been studied. A single tri-allelomorphic individual in Paratettix may be due to autosomal non-disjunction.

The occurrence of supernumerary Y chromosomes and of triploid or multiple chromosomes in various forms can likewise be explained as the result of non-disjunction.

The genetic and cytological evidence in the case of non-disjunction leaves no escape from the conclusion that the X chromosomes are the carriers of the genes for the sex-linked characters. The distribution of sex-linked genes (as tested by experimental breeding methods) has been demonstrated to be identical, through all the details of a unique process, with the distribution of the X chromosomes (as tested by direct cytological examination). The argument that the cell as a whole possesses the tendency to develop certain characters is completely nullified by the fact that in these cases the cells that produce exceptions are of exactly the same parentage as those which do not produce exceptions, the only difference being the parentage of a particular chromosome, the X. Those eggs which have lost nothing but the X chromosome have completely lost therewith the ability to produce any of the maternal sex-linked characters, and with the introduction of an X from the father these eggs have developed all of the sex-linked characters of the father. Conversely, those eggs which have retained both X's of the mother and have received no X from the father show all of the sex-linked characters of the mother and none from the father. The breach which GREGORY found in his case of the tetraploid Primula, namely, that the doubling of

the genes is only an expression of the doubleness of the *cell-as-a-whole,* becomes in this case the strongest bulwark; for here the cell as a whole remains constant and the issue is restricted to *particular* chromosomes and a *particular* class of genes.

Experimental proof is given that particular chromosomes, the X chromosomes, are the differentiators of sex; the X chromosome constitution of an individual is the cause of the development by that individual of a particular sex, and is not the result of sex already determined by some other agent. The sex is not determined in the egg or the sperm as such, but is determined at the moment of fertilization; for the X sperm of a male gives rise to a female when it fertilizes an egg containing an X, but to a male if it fertilizes an egg containing a Y or no sex chromosome at all. Likewise the Y sperm of a male gives rise to a female when fertilizing an XX egg and to a male when fertilizing an X egg. These facts in connection with the fact that an X egg of a female produces a male if fertilized by an X sperm prove that the segregation of the X chromosomes is the segregation of the sex-differentiators. The presence of two X chromosomes determines that an individual shall be a female, the presence of one X that the individual shall be a male. The origin of these chromosomes whether maternal or paternal is without significance in the production of sex.

The Y chromosome is without effect upon the sex or the characters of the individual, for males may have one Y, two Y's, or may lack Y entirely (males lacking Y are sterile); and females may have one or two supernumerary Y's with no change in appearance in any case.

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